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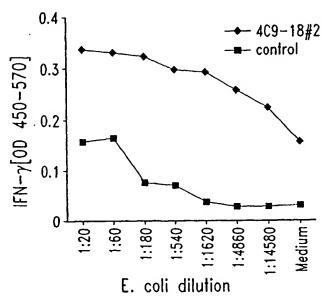
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[Continued on next page]

(54) Title: COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION



(57) Abstract: Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a *Chlamydia* antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.



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COMPOUNDS AND METHODS FOR TREATMENT AND DIAGNOSIS OF CHLAMYDIAL INFECTION

TECHNICAL FIELD

The present invention relates generally to the detection and treatment of Chlamydial infection. In particular, the invention is related to polypeptides comprising a *Chlamydia* antigen and the use of such polypeptides for the serodiagnosis and treatment of Chlamydial infection.

BACKGROUND OF THE INVENTION

Chlamydiae are intracellular bacterial pathogens that are responsible for a wide variety of important human and animal infections. Chlamydia trachomatis is 10 one of the most common causes of sexually transmitted diseases and can lead to pelvic inflammatory disease (PID), resulting in tubal obstruction and infertility. Chlamydia trachomatis may also play a role in male infertility. In 1990, the cost of treating PID in the US was estimated to be \$4 billion. Trachoma, due to ocular infection with Chlamydia trachomatis, is the leading cause of preventable blindness worldwide. 15 Chlamydia pneumonia is a major cause of acute respiratory tract infections in humans and is also believed to play a role in the pathogenesis of atherosclerosis and, in particular, coronary heart disease. Individuals with a high titer of antibodies to Chlamydia pneumonia have been shown to be at least twice as likely to suffer from Chlamydial infections thus coronary heart disease as seronegative individuals. 20 constitute a significant health problem both in the US and worldwide.

Chlamydial infection is often asymptomatic. For example, by the time a woman seeks medical attention for PID, irreversible damage may have already occurred resulting in infertility. There thus remains a need in the art for improved vaccines and pharmaceutical compositions for the prevention and treatment of *Chlamydia* infections. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

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The present invention provides compositions and methods for the diagnosis and therapy of *Chlamydia* infection. In one aspect, the present invention provides polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, or a variant of such an antigen. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments,, the polypeptide comprises an amino acid

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sequence encoded by a polynucleotide sequence selected from the group consisting of (a) a sequence of SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290; (b) the complements of said sequences; and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions. In specific embodiments, the polypeptides of the present invention comprise at least a portion of a Chlamydial protein that includes an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 5-14, 17-20, 26, 28, 30-32, 34, 39-43, 65, 89-109, 138-158, 167, 168, 224-262, 246, 247, 254-256, 292, 294-305 and variants thereof.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of a Chlamydial protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

In a related aspect, polynucleotide sequences encoding the above polypeptides, recombinant expression vectors comprising one or more of these polynucleotide sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising an inventive polypeptide, or, alternatively, an inventive polypeptide and a known Chlamydia antigen, as well as polynucleotides encoding such fusion proteins, in combination with a physiologically acceptable carrier or immunostimulant for use as pharmaceutical compositions and vaccines thereof.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody, both polyclonal and monoclonal, or antigen-binding fragment thereof that specifically binds to a Chlamydial protein; and (b) a physiologically acceptable carrier. Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more Chlamydia polypeptides disclosed herein, or a polynucleotide molecule encoding such a polypeptide, and a The invention also provides vaccines for physiologically acceptable carrier. prophylactic and therapeutic purposes comprising one or more of the disclosed polypeptides and an immunostimulant, as defined herein, together with vaccines comprising one or more polynucleotide sequences encoding such polypeptides and an immunostimulant.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions or vaccines.

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In yet a further aspect, methods for the treatment of Chlamydia infection in a patient are provided, the methods comprising obtaining peripheral blood mononuclear cells (PBMC) from the patient, incubating the PBMC with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated T cells and administering the incubated T cells to the patient. The present invention additionally provides methods for the treatment of Chlamydia infection that comprise incubating antigen presenting cells with a polypeptide of the present invention (or a polynucleotide that encodes such a polypeptide) to provide incubated antigen presenting cells and administering the incubated antigen presenting Proliferated cells may, but need not, be cloned prior to cells to the patient. administration to the patient. In certain embodiments, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages, monocytes, B-cells, and fibroblasts. Compositions for the treatment of Chlamydia infection comprising T cells or antigen presenting cells that have been incubated with a polypeptide or polynucleotide of the present invention are also provided. Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) an immunostimulant.

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The present invention further provides, within other aspects, methods for removing *Chlamydial*-infected cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a *Chlamydial* protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

Within related aspects, methods are provided for inhibiting the development of *Chlamydial* infection in a patient, comprising administering to a patient a biological sample treated as described above. In further aspects of the subject invention, methods and diagnostic kits are provided for detecting *Chlamydia* infection in a patient. In one embodiment, the method comprises: (a) contacting a biological sample with at least one of the polypeptides or fusion proteins disclosed herein; and (b) detecting in the sample the presence of binding agents that bind to the polypeptide or fusion protein, thereby detecting *Chlamydia* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. In one embodiment, the diagnostic kits comprise one or more of the polypeptides or fusion proteins disclosed herein in combination with a detection reagent. In yet another embodiment, the diagnostic kits comprise either a monoclonal antibody or a polyclonal antibody that binds with a polypeptide of the present invention.

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The present invention also provides methods for detecting Chlamydia infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous nucleotides of a polynucleotide sequence peptide disclosed herein, or of a sequence that hybridizes thereto.

In a further aspect, the present invention provides a method for detecting Chlamydia infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a polynucleotide sequence disclosed herein; and (c) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe. embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence disclosed herein, or a sequence that hybridizes thereto.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

SEQUENCE IDENTIFIERS

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SEQ ID NO: 1 is the determined DNA sequence for the C. trachomatis clone 1-B1-66.

SEQ ID NO: 2 is the determined DNA sequence for the C. trachomatis clone 4-D7-28.

SEQ ID NO: 3 is the determined DNA sequence for the C. trachomatis clone 3-G3-10.

SEQ ID NO: 4 is the determined DNA sequence for the C. trachomatis clone 10-C10-31. 30

SEQ ID NO: 5 is the predicted amino acid sequence for 1-B1-66.

SEQ ID NO: 6 is the predicted amino acid sequence for 4-D7-28.

SEQ ID NO: 7 is a first predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 8 is a second predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 9 is a third predicted amino acid sequence for 3-G3-10.

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alu iu nu.	IV IS a IUUIUI	producted airii	no acia sequen	ce for 3-G3-10

SEQ ID NO: 11 is a fifth predicted amino acid sequence for 3-G3-10.

SEQ ID NO: 12 is the predicted amino acid sequence for 10-C10-31.

SEQ ID NO: 13 is the amino acid sequence of the synthetic peptide 1-

5 B1-66/48-67.

SEQ ID NO: 14 is the amino acid sequence of the synthetic peptide 1-B1-66/58-77.

SEQ ID NO: 15 is the determined DNA sequence for the $\it C.$ trachomatis serovar LGV II clone 2C7-8

SEQ ID NO: 16 is a DNA sequence of a putative open reading frame from a region of the C. trachomatis serovar D genome to which 2C7-8 maps

SEQ ID NO: 17 is the predicted amino acid sequence encoded by the DNA sequence of SEQ ID NO: 16

SEQ ID NO: 18 is the amino acid sequence of the synthetic peptide

15 CtC7.8-12

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SEQ ID NO: 19 is the amino acid sequence of the synthetic peptide CtC7.8-13

SEQ ID NO: 20 is the predicted amino acid sequence encoded by a second putative open reading from C. trachomatis serovar D

SEQ ID NO: 21 is the determined DNA sequence for clone 4C9-18 from C. trachomatis LGV II

SEQ ID NO: 22 is the determined DNA sequence homologous to Lipoamide Dehydrogenase from C. trachomatis LGV II

SEQ ID NO: 23 is the determined DNA sequence homologous to Hypothetical protein from C. trachomatis LGV II

SEQ ID NO: 24 is the determined DNA sequence homologous to Ubiquinone Mehtyltransferase from *C. trachomatis* LGV II

SEQ ID NO: 25 is the determined DNA sequence for clone 4C9-18#2 BL21 pLysS from C. trachomatis LGV II

SEQ ID NO: 26 is the predicted amino acid sequence for 4C9-18#2 from C. trachomatis LGV II

SEQ ID NO: 27 is the determined DNA sequence for Cp-SWIB from C. pneumonia strain TWAR

SEQ ID NO: 28 is the predicted amino acid sequence for Cp-SWIB from C. pneumonia strain TWAR

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SEQ ID NO: 29 is the determined DNA sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 30 is the predicted amino acid sequence for Cp-S13 from C. pneumonia strain TWAR

SEQ ID NO: 31 is the amino acid sequence for a 10mer consensus peptide from CtC7.8-12 and CtC7.8-13

SEQ ID NO: 32 is the predicted amino acid sequence for clone 2C7-8 from C. trachomatis LGV II

SEQ ID NO: 33 is the DNA sequence corresponding to nucleotides 597304-597145 of the C. trachomatis serovar D genome (NCBI, BLASTN search), 10 which shows homology to clone 2C7-8

SEQ ID NO: 34 is the predicted amino acid sequence encoded by the sequence of SEQ ID NO: 33

SEQ ID NO: 35 is the DNA sequence for C.p. SWIB Nde (5' primer)

from C. pneumonia 15

SEQ ID NO: 36 is the DNA sequence for C.p. SWIB EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 37 is the DNA sequence for C.p. S13 Nde (5' primer) from C. pneumonia

SEQ ID NO: 38 is the DNA sequence for C.p. S13 EcoRI (3' primer) from C. pneumonia

SEQ ID NO: 39 is the amino acid sequence for CtSwib 52-67 peptide from C. trachomatis LGV II

SEQ ID NO: 40 is the amino acid sequence for CpSwib 53-68 peptide from C. pneumonia 25

SEQ ID NO: 41 is the amino acid sequence for HuSwib 288-302 peptide from Human SWI domain

SEQ ID NO: 42 is the amino acid sequence for CtSWI-T 822-837 peptide from the topoisomerase-SWIB fusion of C. trachomatis

SEQ ID NO: 43 is the amino acid sequence for CpSWI-T 828-842 peptide from the topoisomerase-SWIB fusion of C. pneumonia

SEQ ID NO: 44 is a first determined DNA sequence for the C. trachomatis LGV II clone 19783.3, jen.seq(1>509)CTL2#11-3', representing the 3' end.

SEQ ID NO: 45 is a second determined DNA sequence for the C.

trachomatis LGV II clone 19783.4, jen.seq(1>481)CTL2#11-5', representing the 5' end. 35

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SEQ ID NO: 46 is the determined DNA sequence for the *C. trachomatis* LGV II clone19784CTL2 12consensus.seq(1>427)CTL2#12.

SEQ ID NO: 47 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19785.4,jen.seq(1>600)CTL2#16-5', representing the 5' end.

SEQ ID NO: 48 is a first determined DNA sequence for the C. trachomatis LGV II clone 19786.3 jen.seq(1>600)CTL2#18-3', representing the 3' end.

SEQ ID NO: 49 is a second determined DNA sequence for the C. trachomatis LGV II clone 19786.4,jen.seq(1>600)CTL2#18-5', representing the 5' end.

SEQ ID NO: 50 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19788CTL2_21consensus.seq(1>406)CTL2#21.

SEQ ID NO: 51 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19790CTL2_23consensus.seq(1>602)CTL2#23.

SEQ ID NO: 52 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19791CTL2_24consensus.seq(1>145)CTL2#24.

SEQ ID NO: 53 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#4.

SEQ ID NO: 54 is the determined DNA sequence for the *C. trachomatis* LGV II clone CTL2#8b.

SEQ ID NO: 55 is the determined DNA sequence for the *C. trachomatis* 20 LGV II clone15-G1-89, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 56 is the determined DNA sequence for the *C. trachomatis* LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 57 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-G3-83, sharing homology to the hypothetical protein CT622.

SEQ ID NO: 58 is the determined DNA sequence for the *C. trachomatis* LGV II clone 12-B3-95, sharing homology to the lipoamide dehydrogenase gene CT557.

SEQ ID NO: 59 is the determined DNA sequence for the *C. trachomatis* 30 LGV II clone 11-H4-28, sharing homology to the dnaK gene CT396.

SEQ ID NO: 60 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-H3-68, sharing partial homology to the PGP6-D virulence protein and L1 ribosomal gene CT318.

SEQ ID NO: 61 is the determined DNA sequence for the *C. trachomatis*LGV II clone 11-G1-34, sharing partial homology to the malate dehydrogenase gene

CT376 and to the glycogen hydrolase gene CT042.

SEQ ID NO: 62 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-G10-46, sharing homology to the hypothetical protein CT610.

SEQ ID NO: 63 is the determined DNA sequence for the C. trachomatis LGV II clone 11-C12-91, sharing homology to the OMP2 gene CT443.

SEQ ID NO: 64 is the determined DNA sequence for the *C. trachomatis* LGV II clone 11-A3-93, sharing homology to the HAD superfamily gene CT103.

SEQ ID NO: 65 is the determined amino acid sequence for the C. trachomatis LGV II clone 14-H1-4, sharing homology to the thiol specific antioxidant gene CT603.

SEQ ID NO: 66 is the determined DNA sequence for the *C. trachomatis*LGV II clone CtL2#9.

SEQ ID NO: 67 is the determined DNA sequence for the *C. trachomatis* LGV II clone CtL2#7.

SEQ ID NO: 68 is the determined DNA sequence for the *C. trachomatis*15. LGV II clone CtL2#6.

SEQ ID NO: 69 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#5.

SEQ ID NO: 70 is the determined DNA sequence for the *C. trachomatis* LGV II clone CtL2#2.

SEQ ID NO: 71 is the determined DNA sequence for the C. trachomatis LGV II clone CtL2#1.

SEQ ID NO: 72 is a first determined DNA sequence for the C. trachomatis LGV II clone 23509.2CtL2#3-5', representing the 5' end.

SEQ ID NO: 73 is a second determined DNA sequence for the C. trachomatis LGV II clone 23509.1CtL2#3-3', representing the 3' end.

SEQ ID NO: 74 is a first determined DNA sequence for the C. trachomatis LGV II clone 22121.2CtL2#10-5', representing the 5' end.

SEQ ID NO: 75 is a second determined DNA sequence for the C. trachomatis LGV II clone 22121.1CtL2#10-3', representing the 3' end.

SEQ ID NO: 76 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19787.6CtL2#19-5', representing the 5' end.

SEQ ID NO: 77 is the determined DNA sequence for the *C. pneumoniae* LGV II clone CpS13-His.

SEQ ID NO: 78 is the determined DNA sequence for the C: pneumoniae LGV II clone Cp_SWIB-His.

SEQ ID NO: 79 is the determined DNA sequence for the *C. trachomatis* LGV II clone 23-G7-68, sharing partial homology to the L11, L10 and L1 ribosomal protein.

SEQ ID NO: 80 is the determined DNA sequence for the *C. trachomatis* LGV II clone 22-F8-91, sharing homology to the pmpC gene.

SEQ ID NO: 81 is the determined DNA sequence for the *C. trachomatis* LGV II clone 21-E8-95, sharing homology to the CT610-CT613 genes.

SEQ ID NO: 82 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19-F12-57, sharing homology to the CT858 and recA genes.

SEQ ID NO: 83 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19-F12-53, sharing homology to the CT445 gene encoding glutamyl tRNA synthetase.

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SEQ ID NO: 84 is the determined DNA sequence for the *C. trachomatis* LGV II clone 19-A5-54, sharing homology to the cryptic plasmid gene.

SEQ ID NO: 85 is the determined DNA sequence for the *C. trachomatis* LGV II clone 17-E11-72, sharing partial homology to the OppC_2 and pmpD genes.

SEQ ID NO: 86 is the determined DNA sequence for the *C. trachomatis* LGV II clone 17-C1-77, sharing partial homology to the CT857 and CT858 open reading frames.

SEQ ID NO: 87 is the determined DNA sequence for the *C. trachomatis* LGV II clone 15-H2-76, sharing partial homology to the pmpD and SycE genes, and to the CT089 ORF.

SEQ ID NO: 88 is the determined DNA sequence for the *C. trachomatis* LGV II clone 15-A3-26, sharing homology to the CT858 ORF.

SEQ ID NO: 89 is the determined amino acid sequence for the C. pnuemoniae clone Cp_SWIB-His.

SEQ ID NO: 90 is the determined amino acid sequence for the C. trachomatis LGV II clone CtL2_LPDA_FL.

SEQ ID NO: 91 is the determined amino acid sequence for the C. pnuemoniae clone CpS13-His.

SEQ ID NO: 92 is the determined amino acid sequence for the C. trachomatis LGV II clone CtL2_TSA_FL.

SEQ ID NO: 93 is the amino acid sequence for Ct-Swib 43-61 peptide from C. trachomatis LGV II.

SEQ ID NO: 94 is the amino acid sequence for Ct-Swib 48-67 peptide from C. trachomatis LGV II.

SEQ ID NO: 95 is the amino acid sequence for Ct-Swib 52-71 peptide from C. trachomatis LGV II.

SEQ ID NO: 96 is the amino acid sequence for Ct-Swib 58-77 peptide from C. trachomatis LGV II.

SEQ ID NO: 97 is the amino acid sequence for Ct-Swib 63-82 peptide from C. trachomatis LGV II.

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SEQ ID NO: 98 is the amino acid sequence for Ct-Swib 51-66 peptide from C. trachomatis LGV II.

SEQ ID NO: 99 is the amino acid sequence for Cp-Swib 52-67 peptide from C. pneumonia.

SEQ ID NO: 100 is the amino acid sequence for Cp-Swib 37-51 peptide from *C. pneumonia*.

SEQ ID NO: 101 is the amino acid sequence for Cp-Swib 32-51 peptide from C. pneumonia.

SEQ ID NO: 102 is the amino acid sequence for Cp-Swib 37-56 peptide from *C. pneumonia*.

SEQ ID NO: 103 is the amino acid sequence for Ct-Swib 36-50 peptide from C. trachomatis.

SEQ ID NO: 104 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 105 is the amino acid sequence for Ct-S13 60-80 peptide from C. trachomatis.

SEQ ID NO: 106 is the amino acid sequence for Ct-S13 1-20 peptide from C. trachomatis.

SEQ ID NO: 107 is the amino acid sequence for Ct-S13 46-65 peptide from C. trachomatis.

SEQ ID NO: 108 is the amino acid sequence for Ct-S13 56-75 peptide from C. trachomatis.

SEQ ID NO: 109 is the amino acid sequence for Cp-S13 56-75 peptide 30 from C. pneumoniae.

SEQ ID NO: 110 is the determined DNA sequence for the C. trachomatis LGV II clone 21-G12-60, containing partial open reading frames for hypothetical proteins CT875, CT229 and CT228.

SEQ ID NO: 111 is the determined DNA sequence for the C. 35 trachomatis LGV II clone 22-B3-53, sharing homology to the CT110 ORF of GroEL.

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SEQ ID NO: 112 is the determined DNA sequence for the *C. trachomatis* LGV II clone 22-A1-49, sharing partial homology to the CT660 and CT659 ORFs.

SEQ ID NO: 113 is the determined DNA sequence for the C. trachomatis LGV II clone 17-E2-9, sharing partial homology to the CT611 and CT 610 ORFs.

SEQ ID NO: 114 is the determined DNA sequence for the C. trachomatis LGV II clone 17-C10-31, sharing partial homology to the CT858 ORF.

SEQ ID NO: 115 is the determined DNA sequence for the C. 10 trachomatis LGV II clone 21-C7-66, sharing homology to the dnaK-like gene.

SEQ ID NO: 116 is the determined DNA sequence for the C. trachomatis LGV II clone 20-G3-45, containing part of the pmpB gene CT413.

SEQ ID NO: 117 is the determined DNA sequence for the C. trachomatis LGV II clone 18-C5-2, sharing homology to the S1 ribosomal protein ORF.

SEQ ID NO: 118 is the determined DNA sequence for the *C. trachomatis* LGV II clone 17-C5-19, containing part of the ORFs for CT431 and CT430.

SEQ ID NO: 119 is the determined DNA sequence for the *C. trachomatis* LGV II clone 16-D4-22, contains partial sequences of ORF3 and ORF4 of the plasmid for growth within mammalian cells.

SEQ ID NO: 120 is the determined full-length DNA sequence for the *C. trachomatis* serovar LGV II Cap1 gene CT529.

SEQ ID NO: 121 is the predicted full-length amino acid sequence for the C. trachomatis serovar LGV II Cap1 gene CT529.

SEQ ID NO: 122 is the determined full-length DNA sequence for the *C. trachomatis* serovar E Cap1 gene CT529.

SEQ ID NO: 123 is the predicted full-length amino acid sequence for the C. trachomatis serovar E Cap1 gene CT529.

SEQ ID NO: 124 is the determined full-length DNA sequence for the C. trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 125 is the predicted full-length amino acid sequence for the C. trachomatis serovar 1A Cap1 gene CT529.

SEQ ID NO: 126 is the determined full-length DNA sequence for the *C. trachomatis* serovar G Cap1 gene CT529.

SEQ ID NO: 127 is the predicted full-length amino acid sequence for the C. trachomatis serovar G Cap1 gene CT529.

SEQ ID NO: 128 is the determined full-length DNA sequence for the *C. trachomatis* serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 129 is the predicted full-length amino acid sequence for the C. trachomatis serovar F1 NII Cap1 gene CT529.

SEQ ID NO: 130 is the determined full-length DNA sequence for the *C. trachomatis* serovar L1 Cap1 gene CT529.

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SEQ ID NO: 131 is the predicted full-length amino acid sequence for the C. trachomatis serovar L1 Cap1 gene CT529.

SEQ ID NO: 132 is the determined full-length DNA sequence for the C. trachomatis serovar L3 Cap1 gene CT529.

SEQ ID NO: 133 is the predicted full-length amino acid sequence for the C. trachomatis serovar L3 Cap1 gene CT529.

SEQ ID NO: 134 is the determined full-length DNA sequence for the *C. trachomatis* serovar Ba Cap1 gene CT529.

SEQ ID NO: 135 is the predicted full-length amino acid sequence for the C. trachomatis serovar Ba Cap1 gene CT529.

SEQ ID NO: 136 is the determined full-length DNA sequence for the *C. trachomatis* serovar MOPN Cap1 gene CT529.

SEQ ID NO: 137 is the predicted full-length amino acid sequence for the 20 C. trachomatis serovar MOPN Cap1 gene CT529.

SEQ ID NO: 138 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #124-139 of *C. trachomatis* serovar L2.

SEQ ID NO: 139 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #132-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 140 is the determined amino acid sequence for the Capl CT529 ORF peptide #138-155 of C. trachomatis serovar L2.

SEQ ID NO: 141 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #146-163 of *C. trachomatis* serovar L2.

SEQ ID NO: 142 is the determined amino acid sequence for the Capl CT529 ORF peptide #154-171 of *C. trachomatis* serovar L2.

SEQ ID NO: 143 is the determined amino acid sequence for the Capl CT529 ORF peptide #162-178 of *C. trachomatis* serovar L2.

SEQ ID NO: 144 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-147 of C. trachomatis serovar L2.

SEQ ID NO: 145 is the determined amino acid sequence for the Capl CT529 ORF peptide #139-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 146 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #140-147 of *C. trachomatis* serovar L2.

SEQ ID NO: 147 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-146 of *C. trachomatis* serovar L2.

SEQ ID NO: 148 is the determined amino acid sequence for the Cap1 CT529 ORF peptide #138-145 of *C. trachomatis* serovar L2.

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SEQ ID NO: 149 is the determined amino acid sequence for the Cap1 CT529 ORF peptide # F140->I of C. trachomatis serovar L2.

SEQ ID NO: 150 is the determined amino acid sequence for the Capl 10 CT529 ORF peptide ##S139>Ga of C. trachomatis serovar L2.

SEQ ID NO: 151 is the determined amino acid sequence for the Capl CT529 ORF peptide ##S139>Gb of C. trachomatis serovar L2.

SEQ ID NO: 152 is the determined amino acid sequence for the peptide # 2 C7.8-6 of the 216aa ORF of *C. trachomatis* serovar L2.

SEQ ID NO: 153 is the determined amino acid sequence for the peptide # 2 C7.8-7 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 154 is the determined amino acid sequence for the peptide # 2 C7.8-8 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 155 is the determined amino acid sequence for the peptide 20 #2 C7.8-9 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 156 is the determined amino acid sequence for the peptide # 2 C7.8-10 of the 216aa ORF of C. trachomatis serovar L2.

SEQ ID NO: 157 is the determined amino acid sequence for the 53 amino acid residue peptide of the 216aa ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 158 is the determined amino acid sequence for the 52 amino acid residue peptide of the CT529 ORF within clone 2C7.8 of *C. trachomatis* serovar L2.

SEQ ID NO: 159 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 160 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar L2.

SEQ ID NO: 161 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 for serovars other than L2 and MOPN.

SEQ ID NO: 162 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovars other than L2 and MOPN.

SEQ ID NO: 163 is the determined DNA sequence for the 5' (forward) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 164 is the determined DNA sequence for the 5' (reverse) primer for cloning full-length CT529 serovar MOPN.

SEQ ID NO: 165 is the determined DNA sequence for the 5' (forward) primer for pBIB-KS.

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SEQ ID NO: 166 is the determined DNA sequence for the 5' (reverse) primer for pBIB-KS.

SEQ ID NO: 167 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar L2.

SEQ ID NO: 168 is the determined amino acid sequence for the 9-mer epitope peptide Cap1#139-147 from serovar D.

SEQ ID NO: 169 is the determined full-length DNA sequence for the \mathcal{C} . trachomatis pmpI gene.

SEQ ID NO: 170 is the determined full-length DNA sequence for the C. 15 trachomatis pmpG gene.

SEQ ID NO: 171 is the determined full-length DNA sequence for the C. trachomatis pmpE gene.

SEQ ID NO: 172 is the determined full-length DNA sequence for the C. trachomatis pmpD gene. 20

SEQ ID NO: 173 is the determined full-length DNA sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 174 is the determined full-length DNA sequence for the C. trachomatis pmpB gene.

SEQ ID NO: 175 is the predicted full-length amino acid sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 176 is the predicted full-length amino acid sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 177 is the predicted full-length amino acid sequence for the C. trachomatis pmpE gene. 30

SEQ ID NO: 178 is the predicted full-length amino acid sequence for the C. trachomatis pmpD gene.

SEQ ID NO: 179 is the predicted full-length amino acid sequence for the C. trachomatis pmpC gene.

SEQ ID NO: 180 is the predicted full-length amino acid sequence for the 35 C. trachomatis pmpB gene.

SEQ ID NO: 181 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpI gene.

SEQ ID NO: 182 is a subsequently determined full-length DNA sequence for the C. trachomatis pmpG gene.

SEQ ID NO: 183 is the determined DNA sequence minus the signal sequence for the C. trachomatis pmpE gene.

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SEQ ID NO: 184 is a first determined DNA sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 185 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 186 is a first determined DNA sequence representing the carboxy terminus for the C. trachomatis pmpC gene.

SEQ ID NO: 187 is a second determined DNA sequence representing the amino terminus minus the signal sequence for the *C. trachomatis* pmpC gene.

SEQ ID NO: 188 is the determined DNA sequence representing the *C. pneumoniae* serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 189 is the predicted amino acid sequence minus the signal sequence for the *C. trachomatis* pmpI gene.

SEQ ID NO: 190 is subsequently predicted amino acid sequence for the 20 C. trachomatis pmpG gene.

SEQ ID NO: 191 is the predicted amino acid sequence minus the signal sequence for the *C. trachomatis* pmpE gene.

SEQ ID NO: 192 is a first predicted amino acid sequence representing the carboxy terminus for the *C. trachomatis* pmpD gene.

SEQ ID NO: 193 is a second predicted amino acid sequence representing the Amino terminus minus the signal sequence for the *C. trachomatis* pmpD gene.

SEQ ID NO: 194 is a first predicted amino acid sequence representing the Carboxy terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 195 is a second predicted amino acid sequence representing the Amino terminus for the *C. trachomatis* pmpC gene.

SEQ ID NO: 196 is the predicted amino acid sequence representing the *C. pneumoniae* serovar MOMPS pmp gene in a fusion molecule with Ra12.

SEQ ID NO: 197 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 198 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

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SEQ ID NO: 199 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpC gene in the SKB vaccine vector.

SEQ ID NO: 200 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 201 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

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SEQ ID NO: 202 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpD gene in the SKB vaccine vector.

SEQ ID NO: 203 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.

SEQ ID NO: 204 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the SKB vaccine vector.

SEQ ID NO: 205 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 206 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 207 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 208 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 209 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpC gene in the pET17b vector.

SEQ ID NO: 210 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the C. trachomatis pmpC gene in the pET17b vector.

SEQ ID NO: 211 is the determined DNA sequence for the 5' oligo primer for cloning the amino terminus portion of the C. trachomatis pmpD gene in the pET17b vector.

SEQ ID NO: 212 is the determined DNA sequence for the 3' oligo primer for cloning the amino terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 213 is the determined DNA sequence for the 5' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 214 is the determined DNA sequence for the 3' oligo primer for cloning the carboxy terminus portion of the *C. trachomatis* pmpD gene in the pET17b vector.

SEQ ID NO: 215 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 216 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 217 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpE gene in the pET17b vector.

SEQ ID NO: 218 is the amino acid sequence for the insertion sequence for cloning the *C. trachomatis* pmpE gene in the pET17b vector.

SEQ ID NO: 219 is the determined DNA sequence for the 5' oligo primer for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

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SEQ ID NO: 220 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpG gene in the pET17b vector.

SEQ ID NO: 221 is the amino acid sequence for the insertion sequence for cloning the *C. trachomatis* pmpG gene in the pET17b vector.

SEQ ID NO: 222 is the determined DNA sequence for the 5' oligo primer for cloning the C. trachomatis pmpI gene in the pET17b vector.

SEQ ID NO: 223 is the determined DNA sequence for the 3' oligo primer for cloning the C. trachomatis pmpI gene in the pET17b vector.

SEQ ID NO: 224 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 1-20.

SEQ ID NO: 225 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 6-25.

SEQ ID NO: 226 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 12-31.

SEQ ID NO: 227 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 17-36.

SEQ ID NO: 228 is the determined amino acid sequence for the $\it C$. pneumoniae Swib peptide 22-41.

SEQ ID NO: 229 is the determined amino acid sequence for the *C. pneumoniae* Swib peptide 27-46.

SEQ ID NO: 230 is the determined amino acid sequence for the C. pneumoniae Swib peptide 42-61.

SEQ ID NO: 231 is the determined amino acid sequence for the C. pneumoniae Swib peptide 46-65.

SEQ ID NO: 232 is the determined amino acid sequence for the C. pneumoniae Swib peptide 51-70.

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SEQ ID NO: 233 is the determined amino acid sequence for the C. pneumoniae Swib peptide 56-75.

SEQ ID NO: 234 is the determined amino acid sequence for the C. 10 pneumoniae Swib peptide 61-80.

SEQ ID NO: 235 is the determined amino acid sequence for the C. pneumoniae Swib peptide 66-87.

SEQ ID NO: 236 is the determined amino acid sequence for the $\it C$. $\it trachomatis$ OMCB peptide 103-122.

SEQ ID NO: 237 is the determined amino acid sequence for the C. trachomatis OMCB peptide 108-127.

SEQ ID NO: 238 is the determined amino acid sequence for the $\it C$. trachomatis OMCB peptide 113-132.

SEQ ID NO: 239 is the determined amino acid sequence for the C. 20 trachomatis OMCB peptide 118-137.

SEQ ID NO: 240 is the determined amino acid sequence for the C. trachomatis OMCB peptide 123-143.

SEQ ID NO: 241 is the determined amino acid sequence for the C. trachomatis OMCB peptide 128-147.

SEQ ID NO: 242 is the determined amino acid sequence for the C. trachomatis OMCB peptide 133-152.

SEQ ID NO: 243 is the determined amino acid sequence for the C. trachomatis OMCB peptide 137-156.

SEQ ID NO: 244 is the determined amino acid sequence for the C. trachomatis OMCB peptide 142-161.

SEQ ID NO: 245 is the determined amino acid sequence for the C. trachomatis OMCB peptide 147-166.

SEQ ID NO: 246 is the determined amino acid sequence for the $\it C$. trachomatis OMCB peptide 152-171.

SEQ ID NO: 247 is the determined amino acid sequence for the C. trachomatis OMCB peptide 157-176.

SEQ ID NO: 248 is the determined amino acid sequence for the C. trachomatis OMCB peptide 162-181.

SEQ ID NO: 249 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 167-186.

SEQ ID NO: 250 is the determined amino acid sequence for the C. trachomatis OMCB peptide 171-190.

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SEQ ID NO: 251 is the determined amino acid sequence for the *C. trachomatis* OMCB peptide 171-186.

SEQ ID NO: 252 is the determined amino acid sequence for the C. 10 trachomatis OMCB peptide 175-186.

SEQ ID NO: 252 is the determined amino acid sequence for the C. trachomatis OMCB peptide 175-186.

SEQ ID NO: 253 is the determined amino acid sequence for the C. pneumoniae OMCB peptide 185-198.

SEQ ID NO: 254 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 96-115.

SEQ ID NO: 255 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 101-120.

SEQ ID NO: 256 is the determined amino acid sequence for the C. 20 trachomatis TSA peptide 106-125.

SEQ ID NO: 257 is the determined amino acid sequence for the C. trachomatis TSA peptide 111-130.

SEQ ID NO: 258 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 116-135.

SEQ ID NO: 259 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 121-140.

SEQ ID NO: 260 is the determined amino acid sequence for the *C. trachomatis* TSA peptide 126-145.

SEQ ID NO: 261 is the determined amino acid sequence for the C. 30 trachomatis TSA peptide 131-150.

SEQ ID NO: 262 is the determined amino acid sequence for the C. trachomatis TSA peptide 136-155.

SEQ ID NO: 263 is the determined full-length DNA sequence for the *C. trachomatis* CT529/Cap 1 gene serovar I.

35 SEQ ID NO: 264 is the predicted full-length amino sequence for the *C. trachomatis* CT529/Cap 1 gene serovar I.

SEQ ID NO: 265 is the determined full-length DNA sequence for the C. trachomatis CT529/Cap 1 gene serovar K.

SEQ ID NO: 266 is the predicted full-length amino sequence for the $\it C$. trachomatis CT529/Cap 1 gene serovar $\it K$.

SEQ ID NO: 267 is the determined DNA sequence for the C. trachomatis clone 17-G4-36 sharing homology to part of the ORF of DNA-dirrected RNA polymerase beta subunit- CT315 in serD.

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SEQ ID NO: 268 is the determined DNA sequence for the partial sequence of the $\it C. trachomatis$ CT016 gene in clone 2E10.

SEQ ID NO: 269 is the determined DNA sequence for the partial sequence of the C. trachomatis tRNA syntase gene in clone 2E10.

SEQ ID NO: 270 is the determined DNA sequence for the partial sequence for the C. trachomatis clpX gene in clone 2E10.

SEQ ID NO: 271 is a first determined DNA sequence for the C. trachomatis clone CtL2gam-30 representing the 5'end.

SEQ ID NO: 272 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-30 representing the 3'end.

SEQ ID NO: 273 is the determined DNA sequence for the C. trachomatis clone CtL2gam-28.

SEQ ID NO: 274 is the determined DNA sequence for the C. trachomatis clone CtL2gam-27.

SEQ ID NO: 275 is the determined DNA sequence for the C. trachomatis clone CtL2gam-26.

SEQ ID NO: 276 is the determined DNA sequence for the C. trachomatis clone CtL2gam-24.

SEQ ID NO: 277 is the determined DNA sequence for the C. trachomatis clone CtL2gam-23.

SEQ ID NO: 278 is the determined DNA sequence for the C. trachomatis clone CtL2gam-21.

SEQ ID NO: 279 is the determined DNA sequence for the C. trachomatis clone CtL2gam-18.

SEQ ID NO: 280 is the determined DNA sequence for the $\it C$. trachomatis clone CtL2gam-17.

SEQ ID NO: 281 is a first determined DNA sequence for the C. trachomatis clone CtL2gam-15 representing the 5' end.

SEQ ID NO: 282 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-15 representing the 3' end.

SEQ ID NO: 283 is the determined DNA sequence for the C. trachomatis clone CtL2gam-13.

SEQ ID NO: 284 is the determined DNA sequence for the C. trachomatis clone CtL2gam-10.

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SEQ ID NO: 285 is the determined DNA sequence for the C. trachomatis clone CtL2gam-8.

SEQ ID NO: 286 is a first determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 5' end.

SEQ ID NO: 287 is a second determined DNA sequence for the C. trachomatis clone CtL2gam-6 representing the 3' end.

SEQ ID NO: 288 is the determined DNA sequence for the C. trachomatis clone CtL2gam-5.

SEQ ID NO: 289 is the determined DNA sequence for the C. trachomatis clone CtL2gam-2.

SEQ ID NO: 290 is the determined DNA sequence for the *C. trachomatis* clone CtL2gam-1.

SEQ ID NO: 291 is the determined full-length DNA sequence for the C. pneumoniae homologue of the CT529 gene.

SEQ ID NO: 292 is the predicted full-length amino acid sequence for the *C. pneumoniae* homologue of the CT529 gene.

SEQ ID NO: 293 is the determined DNA sequence for the insertion sequence for cloning the C. trachomatis pmpG gene in the SKB vaccine vector.

SEQ ID NO: 294 is the amino acid sequence of an open reading frame of clone CT603.

SEQ ID NO: 295 is the amino acid sequence of a first open reading frame of clone CT875.

SEQ ID NO: 296 is the amino acid sequence of a second open reading 30 frame of clone CT875.

SEQ ID NO: 297 is the amino acid sequence of a first open reading frame of clone CT858.

SEQ ID NO: 298 is the amino acid sequence of a second open reading frame of clone CT858.

35 SEQ ID NO: 299 is the amino acid sequence of an open reading frame of clone CT622.

- SEQ ID NO: 300 is the amino acid sequence of an open reading frame of clone CT610.
- SEQ ID NO: 301 is the amino acid sequence of an open reading frame of clone CT396.
- SEQ ID NO: 302 is the amino acid sequence of an open reading frame of clone CT318.
 - SEQ ID NO: 304 is the amino acid sequence for C. trachomatis, serovar L2 rCt529c1-125 having a modified N-terminal sequence (6-His tag).
- SEQ ID NO: 305 is the amino acid sequence for C. trachomatis, serovar 10 L2 rCt529c1-125.
 - SEQ ID NO: 306 is the sense primer used in the synthesis of the PmpA(N-term) fusion protein.
 - SEQ ID NO: 307 is the antisense primer used in the synthesis of the PmpA(N-term) fusion protein.
- SEQ ID NO: 308 is the DNA sequence encoding the PmpA(N-term) fusion protein.
 - SEQ ID NO: 309 is the amino acid sequence of the PmpA(N-term) fusion protein.
- SEQ ID NO: 310 is the sense primer used in the synthesis of the 20 PmpA(C-term) fusion protein.
 - SEQ ID NO: 311 is the antisense primer used in the synthesis of the PmpA(C-term) fusion protein.
 - SEQ ID NO: 312 is the DNA sequence encoding the PmpA(C-term) fusion protein.
- SEQ ID NO: 313 is the amino acid sequence of the PmpA(C-term) fusion protein.
 - SEQ ID NO: 314 is the sense primer used in the synthesis of the PmpF(N-term) fusion protein.
- SEQ ID NO: 315 is the antisense primer used in the synthesis of the 30 PmpF(N-term) fusion protein.
 - SEQ ID NO: 316 is the DNA sequence encoding the PmpF(N-term) fusion protein.
 - SEQ ID NO: 317 is the amino acid sequence of the PmpF(N-term) fusion protein.
- SEQ ID NO: 318 is the sense primer used in the synthesis of the PmpF(C-term) fusion protein.

SEQ ID NO: 319 is the antisense primer used in the synthesis of the PmpF(C-term) fusion protein.

SEQ ID NO: 320 is the DNA sequence encoding the PmpF(C-term) fusion protein.

SEQ ID NO: 321 is the amino acid sequence of the PmpF(C-term) fusion protein.

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SEQ ID NO: 322 is the sense primer used in the synthesis of the PmpH(N-term) fusion protein.

SEQ ID NO: 323 is the antisense primer used in the synthesis of the PmpH(N-term) fusion protein.

SEQ ID NO: 324 is the DNA sequence encoding the PmpH(N-term) fusion protein.

SEQ ID NO: 325 is the amino acid sequence of the PmpH(N-term) fusion protein.

SEQ ID NO: 326 is the sense primer used in the synthesis of the PmpH(C-term) fusion protein.

SEQ ID NO: 327 is the antisense primer used in the synthesis of the PmpH(C-term) fusion protein.

SEQ ID NO: 328 is the DNA sequence encoding the PmpH(C-term) fusion protein.

SEQ ID NO: 329 is the amino acid sequence of the PmpH(C-term) fusion protein.

SEQ ID NO: 330 is the sense primer used in the synthesis of the PmpB(1) fusion protein.

SEQ ID NO: 331 is the antisense primer used in the synthesis of the PmpB(1) fusion protein.

SEQ ID NO: 332 is the DNA sequence encoding the PmpB(1) fusion protein.

SEQ ID NO: 333 is the amino acid sequence of the PmpB(1) fusion protein.

SEQ ID NO: 334 is the sense primer used in the synthesis of the PmpB(2) fusion protein.

SEQ ID NO: 335 is the antisense primer used in the synthesis of the PmpB(2) fusion protein.

SEQ ID NO: 336 is the DNA sequence encoding the PmpB(2) fusion protein.

protein.

PmpC(3) fusion protein.

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24 SEQ ID NO: 337 is the amino acid sequence of the PmpB(2) fusion protein. SEQ ID NO: 338 is the sense primer used in the synthesis of the PmpB(3) fusion protein. SEQ ID NO: 339 is the antisense primer used in the synthesis of the PmpB(3) fusion protein. SEQ ID NO: 340 is the DNA sequence encoding the PmpB(3) fusion protein. SEQ ID NO: 341 is the amino acid sequence of the PmpB(3) fusion protein. 10 SEQ ID NO: 342 is the sense primer used in the synthesis of the PmpB(4) fusion protein. SEQ ID NO: 343 is the antisense primer used in the synthesis of the PmpB(4) fusion protein. SEQ ID NO: 344 is the DNA sequence encoding the PmpB(4) fusion 15 protein. SEQ ID NO: 345 is the amino acid sequence of the PmpB(4) fusion protein. SEQ ID NO: 346 is the sense primer used in the synthesis of the PmpC(1) fusion protein. 20 SEQ ID NO: 347 is the antisense primer used in the synthesis of the PmpC(1) fusion protein. SEQ ID NO: 348 is the DNA sequence encoding the PmpC(1) fusion protein. SEQ ID NO: 349 is the amino acid sequence of the PmpC(1) fusion 25 protein. SEQ ID NO: 350 is the sense primer used in the synthesis of the PmpC(2) fusion protein. SEQ ID NO: 351 is the antisense primer used in the synthesis of the PmpC(2) fusion protein. 30 SEQ ID NO: 352 is the DNA sequence encoding the PmpC(2) fusion protein. SEQ ID NO: 353 is the amino acid sequence of the PmpC(2) fusion

SEQ ID NO: 354 is the sense primer used in the synthesis of the

SEQ ID NO: 355 is the antisense primer used in the synthesis of the PmpC(3) fusion protein.

SEQ ID NO: 356 is the DNA sequence encoding the PmpC(3) fusion protein.

SEQ ID NO: 357 is the amino acid sequence of the PmpC(3) fusion protein.

DESCRIPTION OF THE FIGURES

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Fig. 1 illustrates induction of INF-γ from a *Chlamydia*-specific T cell line activated by target cells expressing clone 4C9-18#2.

Fig. 2 illustrates retroviral vectors pBIB-KS1,2,3 modified to contain a Kosak translation initiation site and stop codons.

Fig. 3 shows specific lysis in a chromium release assay of P815 cells pulsed with *Chlamydia* peptides CtC7.8-12 (SEQ ID NO: 18) and CtC7.8-13 (SEQ ID NO: 19).

Fig. 4 shows antibody isotype titers in C57Bl/6 mice immunized with *C. trachomatis* SWIB protein.

Fig. 5 shows *Chlamydia*-specific T-cell proliferative responses in splenocytes from C3H mice immunized with *C. trachomatis* SWIB protein.

Fig. 6 illustrates the 5' and 3' primer sequences designed from C. pneumoniae which were used to isolate the SWIB and S13 genes from C. pneumoniae.

Figs. 7A and 7B show induction of IFN-γ from a human anti-chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia upon activation by monocyte-derived dendritic cells expressing chlamydial proteins.

Fig. 8 shows the identification of T cell epitopes in Chlamydial 25 ribosomal S13 protein with T-cell line TCL 8 EB/DC.

Fig. 9 illustrates the proliferative response of CP-21 T-cells generated against *C. pnuemoniae*-infected dendritic cells to recombinant *C. pneumonia-* SWIBprotein, but not *C. trachomatis* SWIB protein.

Fig. 10 shows the *C. trachomatis*-specific SWIB proliferative responses of a primary T-cell line (TCT-10 EB) from an asymptomatic donor.

Fig. 11 illustrates the identification of T-cell epitope in *C. trachomatis* SWIB with an antigen specific T-cell line (TCL-10 EB).

DETAILED DESCRIPTION OF THE INVENTION

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As noted above, the present invention is generally directed to compositions and methods for the diagnosis and treatment of Chlamydial infection. In one aspect, the compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *Chlamydia* antigen, or a variant thereof.

In specific embodiments, the subject invention discloses polypeptides comprising an immunogenic portion of a *Chlamydia* antigen, wherein the *Chlamydia* antigen comprises an amino acid sequence encoded by a polynucleotide molecule including a sequence selected from the group consisting of (a) nucleotide sequences recited in SEQ ID NO: 1, 15, 21-25, 44-64, 66-76, 79-88, 110-119, 120, 122, 124, 126, 128, 130, 132, 134, 136, 169-174, 181-188, 263, 265 and 267-290 (b) the complements of said nucleotide sequences, and (c) variants of such sequences.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the inventive antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *Chlamydia* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

An "immunogenic portion" of an antigen is a portion that is capable of reacting with sera obtained from a *Chlamydia*-infected individual (i.e., generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). Such immunogenic portions generally comprise at least about 5 amino acid residues, more preferably at least about 10, and

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most preferably at least about 20 amino acid residues. Methods for preparing and identifying immunogenic portions of antigens of known sequence are well known in the art and include those summarized in Paul, Fundamental Immunology, 3rd ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigenspecific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native Chlamydia protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

Examples of immunogenic portions of antigens contemplated by the present invention include, for example, the T cell stimulating epitopes provided in SEQ ID NO: 9, 10, 18, 19, 31, 39, 93-96, 98, 100-102, 106, 108, 138-140, 158, 167, 168, 246, 247 and 254-256. Polypeptides comprising at least an immunogenic portion of one or more *Chlamydia* antigens as described herein may generally be used, alone or in combination, to detect Chlamydial infection in a patient.

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The compositions and methods of the present invention also encompass variants of the above polypeptides and polynucleotide molecules. Such variants include, but are not limited to, naturally occurring allelic variants of the inventive sequences. In particular, variants include other *Chlamydiae* serovars, such as serovars D, E and F, as well as the several LGV serovars which share homology to the inventive polypeptide and polynucleotide molecules described herein. Preferably, the serovar homologues show 95-99% homology to the corresponding polypeptide sequence(s) described herein.

A polypeptide "variant," as used herein, is a polypeptide that differs from the recited polypeptide only in conservative substitutions and/or modifications, such

that the antigenic properties of the polypeptide are retained. In a preferred embodiment, variant polypeptides differ from an identified sequence by substitution, deletion or addition of five amino acids or fewer. Such variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the antigenic properties of the modified polypeptide using, for example, the representative procedures described herein. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (e.g., 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

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As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydropathic nature of the polypeptide. Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the antigenic properties, secondary structure and hydropathic nature of the polypeptide. For example,

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a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

A polynucleotide "variant" is a sequence that differs from the recited nucleotide sequence in having one or more nucleotide deletions, substitutions or additions such that the immunogenicity of the encoded polypeptide is not diminished, relative to the native protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotidedirected site-specific mutagenesis as taught, for example, by Adelman et al. (DNA, 2:183, 1983). Nucleotide variants may be naturally occurring allelic variants as discussed below, or non-naturally occurring variants. The polypeptides provided by the present invention include variants that are encoded by polynucleotide sequences which are substantially homologous to one or more of the polynucleotide sequences "Substantial homology," as used herein, refers to specifically recited herein. polynucleotide sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the event of cross-species homology, at 45°C with 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Such hybridizing polynucleotide sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode a polypeptide that is the same as a polypeptide of the present invention.

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Two nucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Resarch Foundaiton, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) Fast and sensitive multiple sequence alignments on a microcomputer CABIOS 5:151-153; Myers, E.W. and Muller W. (1988) Optimal alignments in linear space CABIOS 4:11-17; Robinson, E.D. (1971) Comb. Theor 11:105; Santou, N. Nes, M. (1987) The neighbor joining method. A new method for reconstructing phylogenetic trees Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) Rapid similarity searches of nucleic acid and protein data banks Proc. Natl. Acad., Sci. USA 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) Add. APL.

20 Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity methods of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (U.S.A.) 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One illustrative example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) Nuc. Acids Res. 25:3389-3402 and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be

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used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or amino acid sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

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Therefore, the present invention provides polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% or more sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (e.g., BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two polynucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides or polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides and polypeptides encompassed by this invention may comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000

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or more contiguous nucleotides of one or more of the disclosed sequences, as well as all intermediate lengths therebetween. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, etc.; 21, 22, 23, etc.; 30, 31, 32, etc.; 50, 51, 52, 53, etc.; 100, 101, 102, 103, etc.; 150, 151, 152, 153, etc.; including all integers through the 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

Also included in the scope of the present invention are alleles of the genes encoding the nucleotide sequences recited in herein. As used herein, an "allele" or "allellic sequence" is an alternative form of the gene which may result from at least one mutation in the nucleic acid sequence. Alleles may result in altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone or in combination with the others, one or more times in a given sequence. In specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a Chlamydia antigen (or a variant of such an antigen), that comprises one or more of the amino acid sequences encoded by (a) a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1-4, 15 21-25, 44-64, 66-76 and 79-88; (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b). As discussed in the Examples below, several of the Chlamydia antigens disclosed herein recognize a T cell line that recognizes both Chlamydia trachomatis and Chlamydia pneumoniae infected monocyte-derived dendritic cells, indicating that they may represent an immunoreactive epitope shared by Chlamydia trachomatis and Chlamydia pneumoniae. The antigens may thus be

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employed in a vaccine for both *C. trachomatis* genital tract infections and for *C. pneumonia* infections. Further characterization of these *Chlamydia* antigens from *Chlamydia trachomatis* and *Chlamydia pneumonia* to determine the extent of cross-reactivity is provided in Example 6. Additionally, Example 4 describes cDNA fragments (SEQ ID NO: 15, 16 and 33) isolated from *C. trachomatis* which encode proteins (SEQ ID NO: 17-19 and 32) capable of stimulating a *Chlamydia*-specific murine CD8+ T cell line.

In general, Chlamydia antigens, and polynucleotide sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, polynucleotide molecules encoding Chlamydia antigens may be isolated from a Chlamydia genomic or cDNA expression library by screening with a Chlamydiaspecific T cell line as described below, and sequenced using techniques well known to those of skill in the art. Additionally, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for Chlamydia-associated expression (i.e., expression that is at least two fold greater in Chlamydia-infected cells than in controls, as determined using a representative assay provided herein). Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., Proc. Natl. Acad. Sci. USA 93:10614-10619, 1996 and Heller et al., Proc. Natl. Acad. Sci. USA 94:2150-2155, 1997). Alternatively, polypeptides may be amplified from cDNA prepared from cells expressing the proteins described herein.. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequencespecific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

Antigens may be produced recombinantly, as described below, by inserting a polynucleotide sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Antigens may be evaluated for a desired property, such as the ability to react with sera obtained from a *Chlamydia*-infected individual as described herein, and may be sequenced using, for example, traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem.* 80:116-132, 1967.

Polynucleotide sequences encoding antigens may also be obtained by screening an appropriate *Chlamydia* cDNA or genomic DNA library for polynucleotide sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold

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Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

An amplified portion may be used to isolate a full length gene from a suitable library (e.g., a Chlamydia cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using techniques well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263, 1987; Erlich ed., PCR Technology, Stockton Press, NY, 1989), and software well known in the art may also be employed. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may

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be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (see Triglia et al., Nucl. Acids Res. 16:8186, 1988), which uses restriction enzymes to generate a fragment in the 5 known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Additional techniques include capture PCR (Lagerstrom et al., PCR Methods Applic. 1:111-19, 1991) and walking PCR (Parker et al., Nucl. Acids. Res. 19:3055-60, 1991). Transcription-Mediated Amplification, or TMA is another method that may be utilized for the amplification of DNA, rRNA, or mRNA, as described in Patent No. PCT/US91/03184. This autocatalytic and isothermic non-PCR based method utilizes two primers and two enzymes: RNA polymerase and reverse transcriptase. One primer contains a promoter sequence for RNA polymerase. In the first amplification, the promoter-primer hybridizes to the target rRNA at a defined site. Reverse transcriptase creates a DNA copy of the target rRNA by extension from the 3'end of the promoterprimer. The RNA in the resulting complex is degraded and a second primer binds to the DNA copy. A new strand of DNA is synthesized from the end of the primer by reverse transcriptase creating double stranded DNA. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the TMA process and serves as a template for a new round of replication leading to the expotential expansion of the RNA amplicon. Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

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In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length cDNA sequences may also be obtained by analysis of genomic fragments.

Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (see Adelman et al., DNA 2:183, 1983). Alternatively, RNA molecules may be generated by in vitro or in vivo transcription of DNA sequences encoding a Chlamydial protein, or portion thereof, provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated in vivo (e.g., by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding a Chlamydial polypeptide, and administering the transfected cells to the patient).

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A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells of tissues to facilitate the production of antisense RNA. An antisense polynucleotide may be used, as described herein, to inhibit expression of a *Chlamydial* protein. Antisense technology can be used to control gene expression through triplehelix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (*see* Gee et al., *In* Huber and Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co. (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

A portion of a coding sequence, or of a complementary sequence, may also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

Any polynucleotide may be further modified to increase stability in vivo. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional

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bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division, Foster City, CA, and may be operated according to the manufacturer's instructions.

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As noted above, immunogenic portions of *Chlamydia* antigens may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for immunogenic properties. The representative ELISAs described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates a signal in such assays that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of a *Chlamydia* antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in a model ELISA as described herein.

Portions and other variants of *Chlamydia* antigens may be generated by synthetic or recombinant means. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the polynucleotide sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a polynucleotide sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides as described herein. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

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In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure.

Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known *Chlamydial* protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein. A DNA sequence encoding a fusion protein of the present invention may be constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding, for example, the first and second polypeptides, into an appropriate expression vector. The 3' end of a DNA

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sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide 10 linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8562, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. As an alternative to the use of a peptide linker sequence (when desired), one can utilize non-essential N-terminal amino acid regions (when present) on the first and second polypeptides to separate the functional domains and prevent steric hindrance.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided that comprise a polypeptide of the present invention together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (see, for example, Stoute et al. New Engl. J. Med., 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium Haemophilus influenza B (WO 91/18926). Preferably, a protein D derivative comprises 10

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approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in E. coli (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

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In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene 43*:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology 10*:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In another embodiment, a Mycobacterium tuberculosis-derived Ra12 polynucleotide is linked to at least an immunogenic portion of a polynucleotide of this invention. Ra12 compositions and methods for their use inenhancing expression of heterologous polynucleotide sequences is described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference in its entirety. Briefly, Ra12 refers to a polynucleotide region that is a subsequence of a *Mycobacterium tuberculosis* MTB32A nucleic acid. MTB32A is a serine protease of 32 KD molecular weight encoded by a gene in virulent and avirulent strains of *M. tuberculosis*. The nucleotide sequence and amino acid sequence of MTB32A have been described (U.S. Patent Application 60/158,585; see also, Skeiky *et al.*, *Infection and Immun.* (1999) 67:3998-4007, incorporated herein by reference. In one embodiment, the Ra12 polypeptide used in the production of fusion polypeptides comprises a C-terminal fragment of the MTB32A coding sequence that is effective for enhancing the

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expression and/or immunogenicity of heterologous Chlamydial antigenic polypeptides with which it is fused. In another embodiment, the Ra12 polypeptide corresponds to an approximately 14 kD. C-terminal fragment of MTB32A comprising some or all of amino acid residues 192 to 323 of MTB32A.

Recombinant nucleic acids, which encode a fusion polypeptide comprising a Ra12 polypeptide and a heterologous Chlamydia polypeptide of interest, can be readily constructed by conventional genetic engineering techniques. Recombinant nucleic acids are constructed so that, preferably, a Ra12 polynucleotide sequence is located 5' to a selected heterologous Chlamydia polynucleotide sequence. It may also be appropriate to place a Ra12 polynucleotide sequence 3' to a selected heterologous polynucleotide sequence or to insert a heterologous polynucleotide sequence into a site within a Ra12 polynucleotide sequence.

In addition, any suitable polynucleotide that encodes a Ra12 or a portion or other variant thereof can be used in constructing recombinant fusion polynucleotides comprising Ra12 and one or more Chlamydia polynucleotides disclosed herein. Preferred Ra12 polynucleotides generally comprise at least about 15 consecutive nucleotides, at least about 30 nucleotides, at least about 60 nucleotides, at least about 100 nucleotides, at least about 200 nucleotides, or at least about 300 nucleotides that encode a portion of a Ra12 polypeptide.

Ra12 polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes a Ra12 polypeptide or a portion thereof) or may comprise a variant of such a sequence. Ra12 polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not substantially diminished, relative to a fusion polypeptide comprising a native Ra12 polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native Ra12 polypeptide or a portion thereof.

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or polynucleotides encoding such polypeptides or fusion proteins) to induce protective immunity against Chlamydial infection in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat Chlamydial infection.

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In this aspect, the polypeptide, fusion protein or polynucleotide molecule is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and an immunostimulant, such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other *Chlamydia* antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

Alternatively, a vaccine may contain polynucleotides encoding one or more polypeptides or fusion proteins as described above, such that the polypeptide is generated in situ. In such vaccines, the polynucleotides may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary polynucleotide sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerrin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the polynucleotides may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve Techniques for incorporating the use of a non-pathogenic (defective) virus. polynucleotides into such expression systems are well known to those of ordinary skill in the art. The polynucleotides may also be administered as "naked" plasmid vectors as described, for example, in Ulmer et al., Science 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The uptake of naked

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polynucleotides may be increased by incorporating the polynucleotides into and/or onto biodegradable beads, which are efficiently transported into the cells. The preparation and use of such systems is well known in the art.

In a related aspect, a polynucleotide vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *Chlamydia* antigen. For example, administration of polynucleotides encoding a polypeptide of the present invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Polypeptides and polynucleotides disclosed herein may also be employed in adoptive immunotherapy for the treatment of *Chlamydial* infection. Adoptive immunotherapy may be broadly classified into either active or passive immunotherapy. In active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system with the administration of immune responsemodifying agents (for example, vaccines, bacterial adjuvants, and/or cytokines).

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In passive immunotherapy, treatment involves the delivery of biologic reagents with established immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate anti-Chlamydia effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocyte, CD4+ T-helper), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Patent No. 4.918,164), for passive immunotherapy.

The predominant method of procuring adequate numbers of T-cells for adoptive immunotherapy is to grow immune T-cells *in vitro*. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. These *in vitro* culture conditions typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast, or B-cells, may be pulsed with immunoreactive polypeptides, or polynucleotide sequence(s) may be introduced into antigen presenting cells, using a variety of standard techniques well known in the art. For example, antigen presenting

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sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. Several viral vectors may be used to transduce an antigen presenting cell, including pox virus, vaccinia virus, and adenovirus; also, antigen presenting cells may be transfected with polynucleotide sequences disclosed herein by a variety of means, including gene-gun technology, lipid-mediated delivery, electroporation, osmotic shock, and particlate delivery mechanisms, resulting in efficient and acceptable expression levels as determined by one of ordinary skill in the art. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term *in vivo*. Studies have demonstrated that cultured T-cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T Cells: Principles Revisited," *Immunological Reviews*, 157:177, 1997).

The polypeptides disclosed herein may also be employed to generate and/or isolate chlamydial-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by *in vivo* immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ or CD4+ T-cell clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides may be employed to generate *Chlamydia* reactive T cell subsets by selective *in vitro* stimulation and expansion of autologous T cells to provide antigenspecific T cells which may be subsequently transferred to the patient as described, for example, by Chang *et al*, (*Crit. Rev. Oncol. Hematol.*, 22(3), 213, 1996). Cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as IsolexTM System, available from Nexell Therapeutics, Inc. Irvine, CA. The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T cells. The population of antigen-specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

In other embodiments, T-cell and/or antibody receptors specific for the polypeptides disclosed herein can be cloned, expanded, and transferred into other vectors or effector cells for use in adoptive immunotherapy. In particular, T cells may

be transfected with the appropriate genes to express the variable domains from chlamydia specific monoclonal antibodies as the extracellular recognition elements and joined to the T cell receptor signaling chains, resulting in T cell activation, specific lysis, and cytokine release. This enables the T cell to redirect its specificity in an MHC-independent manner. See for example, Eshhar, Z., Cancer Immunol Immunother, 45(3-4):131-6, 1997 and Hwu, P., et al, Cancer Res, 55(15):3369-73, 1995. Another embodiment may include the transfection of chlamydia antigen specific alpha and beta T cell receptor chains into alternate T cells, as in Cole, DJ, et al, Cancer Res, 55(4):748-52, 1995.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-specific dendritic cells may either be transferred into a patient, or employed to stimulate T cells to provide antigen-specific T cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T cells and the subsequent use of such antigen-specific T cells to eradicate disease in a murine model has been demonstrated by Cheever et al, *Immunological Reviews*, 157:177, 1997). Additionally, vectors expressing the disclosed polynucleotides may be introduced into stem cells taken from the patient and clonally propagated *in vitro* for autologous transplant back into the same patient.

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Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents disclosed herein may be incorporated into pharmaceutical compositions or immunogenic compositions (i.e., vaccines). Alternatively, a pharmaceutical composition may comprise an antigen-presenting cell (e.g. a dendritic cell) transfected with a Chlamydial polynucleotide such that the antigen presenting cell expresses a Chlamydial polypeptide. Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines may comprise one or more such compounds and an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; see e.g., Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic

portions of other *Chlamydial* antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

A pharmaceutical composition or vaccine may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope.

In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, adenovirus, baculovirus, togavirus, bacteriophage, and the like), which often involves the use of a non-pathogenic (defective), replication competent virus.

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For example, many viral expression vectors are derived from viruses of the retroviridae family. This family includes the murine leukemia viruses, the mouse mammary tumor viruses, the human foamy viruses, Rous sarcoma virus, and the immunodeficiency viruses, including human, simian, and feline. Considerations when designing retroviral expression vectors are discussed in Comstock *et al.* (1997).

Excellent murine leukemia virus (MLV)-based viral expression vectors have been developed by Kim et al. (1998). In creating the MLV vectors, Kim et al. found that the entire gag sequence, together with the immediate upstream region, could be deleted without significantly affecting viral packaging or gene expression. Further, it was found that nearly the entire U3 region could be replaced with the immediately-early promoter of human cytomegalovirus without deleterious effects. Additionally, MCR and internal ribosome entry sites (IRES) could be added without adverse effects. Based on their observations, Kim et al. have designed a series of MLV-based expression vectors comprising one or more of the features described above.

As more has been learned about human foamy virus (HFV), characteristics of HFV that are favorable for its use as an expression vector have been discovered. These characteristics include the expression of pol by splicing and start of

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translation at a defined initiation codon. Other aspects of HFV viral expression vectors are reviewed in Bodem et al. (1997).

Murakami et al. (1997) describe a Rous sarcoma virus (RSV)-based replication-competent avian retrovirus vectors, IR1 and IR2 to express a heterologous gene at a high level. In these vectors, the IRES derived from encephalomyocarditis virus (EMCV) was inserted between the env gene and the heterologous gene. The IR1 vector retains the splice-acceptor site that is present downstream of the env gene while the IR2 vector lacks it. Murakami et al. have shown high level expression of several different heterologous genes by these vectors.

Recently, a number of lentivirus-based retroviral expression vectors have been developed. Kafri et al. (1997) have shown sustained expression of genes delivered directly into liver and muscle by a human immunodeficiency virus (HIV)-based expression vector. One benefit of the system is the inherent ability of HIV to transduce non-dividing cells. Because the viruses of Kafri et al. are pseudotyped with vesicular stomatitis virus G glycoprotein (VSVG), they can transduce a broad range of tissues and cell types.

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A large number of adenovirus-based expression vectors have been developed, primarily due to the advantages offered by these vectors in gene therapy applications. Adenovirus expression vectors and methods of using such vectors are the subject of a number of United States patents, including United States Patent No. 5,698,202, United States Patent No. 5,616,326, United States Patent No. 5,585,362, and United States Patent No. 5,518,913, all incorporated herein by reference.

Additional adenoviral constructs are described in Khatri et al. (1997) and Tomanin et al. (1997). Khatri et al. describe novel ovine adenovirus expression vectors and their ability to infect bovine nasal turbinate and rabbit kidney cells as well as a range of human cell type, including lung and foreskin fibroblasts as well as liver, prostate, breast, colon and retinal lines. Tomanin et al. describe adenoviral expression vectors containing the T7 RNA polymerase gene. When introduced into cells containing a heterologous gene operably linked to a T7 promoter, the vectors were able to drive gene expression from the T7 promoter. The authors suggest that this system may be useful for the cloning and expression of genes encoding cytotoxic proteins.

Poxviruses are widely used for the expression of heterologous genes in mammalian cells. Over the years, the vectors have been improved to allow high expression of the heterologous gene and simplify the integration of multiple heterologous genes into a single molecule. In an effort to diminish cytopathic effects and to increase safety, vaccinia virus mutant and other poxviruses that undergo abortive

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infection in mammalian cells are receiving special attention (Oertli et al., 1997). The use of poxviruses as expression vectors is reviewed in Carroll and Moss (1997).

Togaviral expression vectors, which includes alphaviral expression vectors have been used to study the structure and function of proteins and for protein production purposes. Attractive features of togaviral expression vectors are rapid and efficient gene expression, wide host range, and RNA genomes (Huang, 1996). Also, recombinant vaccines based on alphaviral expression vectors have been shown to induce a strong humoral and cellular immune response with good immunological memory and protective effects (Tubulekas et al., 1997). Alphaviral expression vectors and their use are discussed, for example, in Lundstrom (1997).

In one study, Li and Garoff (1996) used Semliki Forest virus (SFV) expression vectors to express retroviral genes and to produce retroviral particles in BHK-21 cells. The particles produced by this method had protease and reverse transcriptase activity and were infectious. Furthermore, no helper virus could be detected in the virus stocks. Therefore, this system has features that are attractive for its use in gene therapy protocols.

Baculoviral expression vectors have traditionally been used to express heterologous proteins in insect cells. Examples of proteins include mammalian chemokine receptors (Wang et al., 1997), reporter proteins such as green fluorescent protein (Wu et al., 1997), and FLAG fusion proteins (Wu et al., 1997; Koh et al., 1997). Recent advances in baculoviral expression vector technology, including their use in virion display vectors and expression in mammalian cells is reviewed by Possee (1997). Other reviews on baculoviral expression vectors include Jones and Morikawa (1996) and O'Reilly (1997).

Other suitable viral expression systems are disclosed, for example, in Fisher-Hoch et al., Proc. Natl. Acad. Sci. USA 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991; Kolls et al., Proc. Natl. Acad. Sci. USA 91:215-219, 1994; Kass-Eisler et al., Proc. Natl. Acad. Sci. USA 90:11498-11502, 1993; Guzman et al., Circulation 88:2838-2848, 1993; and Guzman et al., Cir. Res. 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. In other systems, the DNA may be introduced as "naked" DNA, as described, for example, in Ulmer et al., Science 35 259:1745-1749, 1993 and reviewed by Cohen, Science 259:1691-1692, 1993. The

uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

It will be apparent that a vaccine may comprise a polynucleotide and/or a polypeptide component, as desired. It will also be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and/or polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts). While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

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Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium tuberculosis derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant

and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, under select circumstances, the adjuvant composition may be designed to induce an immune response predominantly of the Th1 type or Th2 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, Ann. Rev. Immunol. 7:145-173, 1989.

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Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555 and WO 99/33488. Immunostimulatory DNA sequences are also described, for example, by Sato et al., Science 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

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Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa Corporation; Seattle, WA), RC-529 (Corixa Corporation; Seattle, WA) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immunostimulant and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (see, e.g., Coombes et al., Vaccine 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

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Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-coglycolide), as well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (see e.g., U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets *Chlamydia*-infected cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the

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antigen, to improve activation and/or maintenance of the T cell response, to have anti-Chlamydia effects per se and/or to be immunologically compatible with the receiver (i.e., matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, Nature 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic immunity (see Timmerman and Levy, Ann. Rev. Med. 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate in situ, with marked cytoplasmic processes (dendrites) visible in vitro), their ability to take up, process and present antigens with high efficiency, and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells in vivo or ex vivo, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (see Zitvogel et al., Nature Med. 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcy receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and

class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a Chlamydial protein (or portion or other variant thereof) such that the Chlamydial polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place ex vivo, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs in vivo. In vivo and ex vivo transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., Immunology and cell Biology 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the Chlamydial polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

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Routes and frequency of administration of pharmaceutical compositions and vaccines, as well as dosage, will vary from individual to individual. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from *Chlamydial* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

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In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a *Chlamydial* protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

In another aspect, the present invention provides methods for using the polypeptides described above to diagnose Chlamydial infection. In this aspect, methods are provided for detecting Chlamydial infection in a biological sample, using one or more of the above polypeptides, either alone or in combination. For clarity, the term "polypeptide" will be used when describing specific embodiments of the inventive diagnostic methods. However, it will be clear to one of skill in the art that the fusion proteins of the present invention may also be employed in such methods.

As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient. The polypeptides are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to *Chlamydia* antigens which may be indicative of *Chlamydia*-infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (i.e., one component polypeptide will tend to detect infection in samples where the infection would not be detected by another

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component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with *Chlamydia*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested.

A variety of assay formats are known to those of ordinary skill in the art for using one or more polypeptides to detect antibodies in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labeled with a reporter group (e.g., in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labeled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labeled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate, or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

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The polypeptides may be bound to the solid support using a variety of techniques known to those of ordinary skill in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general,

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contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 μ g, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin (BSA) or Tween 20TM (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable dilutent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of antibody within an HGE-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20TM. Detection reagent may then be added to the solid support. An appropriate detection reagent is any

compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known to those in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known to those of ordinary skill in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (e.g., Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

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The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-Chlamydia antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for Chlamydia-infection. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, pp. 106-107. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off

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value on the plot that is the closest to the upper left-hand corner (i.e., the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for Chlamydial infection.

In a related embodiment, the assay is performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (e.g., protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-Chlamydia antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 µg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (e.g., one drop) of patient serum or blood.

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Of course, numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only. One example of an alternative assay protocol which may be usefully employed in such methods is a Western blot, wherein the proteins present in a biological sample are separated on a gel, prior to exposure to a binding agent. Such techniques are well known to those of skill in the art.

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a *Chlamydial* protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically

bind" to a *Chlamydial* protein if it reacts at a detectable level (within, for example, an ELISA) with a *Chlamydial* protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10³ L/mol. The binding constant may be determined using methods well known in the art.

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Binding agents may be further capable of differentiating between patients with and without a *Chlamydial* infection using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a *Chlamydial* protein will generate a signal indicating the presence of a *Chlamydial* infection in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without infection. To determine whether a binding agent satisfies this requirement, biological samples (e.g., blood, sera, sputum urine and/or tissue biopsies) from patients with and without *Chlamydial* infection (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen

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without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, Eur. J. Immunol. 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane,

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Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ⁹⁰Y, ¹²³I, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, and ²¹²Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diptheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

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A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction

of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (e.g., U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

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A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in site-specific regions by appropriate methods. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density, and the rate of clearance of the antibody.

Antibodies may be used in diagnostic tests to detect the presence of *Chlamydia* antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting Chlamydial infection in a patient.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions

thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify *Chlamydia*-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

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As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80%, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis et al. Ibid, Ehrlich, Ibid). Primers or probes may thus be used to detect Chlamydia-specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone or in combination with each other.

The following Examples are offered by way of illustration and not by way of limitation.

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EXAMPLE 1

ISOLATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS

Chlamydia antigens of the present invention were isolated by expression cloning of a genomic DNA library of Chlamydia trachomatis LGV II essentially as described by Sanderson et al. (J. Exp. Med., 1995, 182:1751-1757) and were shown to induce PBMC proliferation and IFN-γ in an immunoreactive T cell line.

A Chlamydia-specific T cell line was generated by stimulating PBMCs from a normal donor with no history of chlamydial genital tract infection with elementary bodies of Chlamydia trachomatis LGV II. This T cell line, referred to as TCL-8, was found to recognize both Chlamydia trachomatis and Chlamydia pneumonia infected monocyte-derived dendritic cells.

A randomly sheared genomic library of *Chlamydia trachomatis* LGV II was constructed in Lambda ZAP (Stratagene, La Jolla, CA) and the amplified library plated out in 96 well microtiter plates at a density of 30 clones/well. Bacteria were induced to express recombinant protein in the presence of 2 mM IPTG for 3 h, then pelleted and resuspended in 200 μl of RPMI 10% FBS. 10 μl of the induced bacterial suspension was transferred to 96 well plates containing autologous monocyte-derived dendritic cells. After a 2 h incubation, dendritic cells were washed to remove free *E. coli* and *Chlamydia*-specific T cells were added. Positive *E. coli* pools were identified by determining IFN-γ production and proliferation of the T cells in response to the pools.

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Four positive pools were identified, which were broken down to yield four pure clones (referred to as 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31), with insert sizes of 481 bp, 183 bp, 110 bp and 1400 bp, respectively. The determined DNA sequences for 1-B1-66, 4-D7-28, 3-G3-10 and 10-C10-31 are provided in SEQ ID NO: 1-4, respectively. Clone 1-B1-66 is approximately in region 536690 of the *C. trachomatis* genome (NCBI *C. trachomatis* database). Within clone 1-B1-66, an open reading frame (ORF) has been identified (nucleotides 115 - 375) that encodes a previously identified 9 kDa protein (Stephens, et al. Genbank Accession No. AE001320), the sequence of which is provided in SEQ ID NO: 5). Clone 4-D7-28 is a smaller region of the same ORF (amino acids 22-82 of 1-B1-66). Clone 3-G3-10 is approximately in region 74559 of the *C. trachomatis* genome. The insert is cloned in the antisense orientation with respect to its orientation in the genome. The clone 10-C10-31 contains an open reading frame that corresponds to a previously published sequence for S13 ribosomal protein from *Chlamydia trachomatis* (Gu, L. et al. *J. Bacteriology*, 177:2594-2601, 1995). The predicted protein sequences for 4-D7-28 and

10-C10-31 are provided in SEQ ID NO: 6 and 12, respectively. Predicted protein sequences for 3-G3-10 are provided in SEQ ID NO: 7-11.

In a related series of screening studies, an additional T cell line was used to screen the genomic DNA library of *Chlamydia trachomatis* LGV II described above. A *Chlamydia*-specific T cell line (TCT-1) was derived from a patient with a chlamydial genital tract infection by stimulating patient PBMC with autologous monocyte-derived dendritic cells infected with elementary bodies of *Chlamydia trachomatis* LGV II. One clone, 4C9-18 (SEQ ID NO: 21), containing a 1256 bp insert, elicited a specific immune response, as measured by standard proliferation assays, from the *Chlamydia*-specific T cell line TCT-1. Subsequent analysis revealed this clone to contain three known sequences: lipoamide dehydrogenase (Genbank Accession No. AE001326), disclosed in SEQ ID NO: 22; a hypothetical protein CT429 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 23; and part of an open reading frame of ubiquinone methyltransferase CT428 (Genbank Accession No. AE001316), disclosed in SEQ ID NO: 24.

In further studies involving clone 4C9-18 (SEQ ID NO: 21), the full-length amino acid sequence for lipoamide dehydrognase (SEQ ID NO: 22) from *C. trachomatis* (LGV II) was expressed in clone CtL2-LPDA-FL, as disclosed in SEQ ID NO: 90.

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To further characterize the open reading frame containing the T cell stimulating epitope(s), a cDNA fragment containing nucleotides 1-695 of clone 4C9-18 with a cDNA sequence encoding a 6X-Histidine tag on the amino terminus was subcloned into the NdeI/EcoRI site of the pET17b vector (Novagen, Madison, WI), referred to as clone 4C9-18#2 BL21 pLysS (SEQ ID NO: 25, with the corresponding amino acid sequence provided in SEQ ID NO: 26) and transformed into E. coli. Selective induction of the transformed E. coli with 2 mM IPTG for three hours resulted in the expression of a 26 kDa protein from clone 4C9-18#2 BL21 pLysS, as evidenced by standard Coomassie-stained SDS-PAGE. To determine the immunogenicity of the protein encoded by clone 4C9-18#2 BL21 pLysS, E. coli expressing the 26 kDa protein were titered onto 1 x 10⁴ monocyte-derived dendritic cells and incubated for two hours. The dendritic cell cultures were washed and 2.5 x 104 T cells (TCT-1) added and allowed to incubate for an additional 72 hours, at which time the level of IFN-y in the culture supernatant was determined by ELISA. As shown in Fig. 1, the T-cell line TCT-1 was found to respond to induced cultures as measured by IFN-g, indicating a Chlamydia-specific T-cell response against the lipoamide dehydrogenase sequence.

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Similarly, the protein encoded by clone 4C9-18#2 BL21 pLysS was shown to stimulate the TCT-1 T-cell line by standard proliferation assays.

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Subsequent studies to identify additional Chlamydia trachomatis antigens using the above-described CD4+ T-cell expression cloning technique yielded additional clones. The TCT-1 and TCL-8 Chlamydia-specific T-cell lines, as well as the TCP-21 T-cell line were utilized to screen the Chlamydia trachomatis LGVII genomic library. The TCP-21 T-cell line was derived from a patient having a humoral immune response to Chlamydia pnuemoniae. The TCT-1 cell line identified 37 positive pools, the TCT-3 cell line identified 41 positive pools and the TCP-21 cell line identified 2 positive pools. The following clones were derived from 10 of these positive pools. Clone 11-A3-93 (SEQ ID NO: 64), identified by the TCP-21 cell line, is a 1339 bp genomic fragment sharing homology to the HAD superfamily (CT103). The second insert in the same clone shares homology with the fab I gene (CT104) present on the complementary strand. Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pnuemoniae.

Clone 11-G10-46, (SEQ ID NO: 62), identified using the TCT-3 cell line, contains a 688 bp insert that shares homology to the hypothetical protein CT610. Clone 11-G1-34, (SEQ ID NO: 61), identified using the TCT-3 cell line, has two partial open reading frames (ORF) with an insert size of 1215 bp. One ORF shares homology to the malate dehydrogenase gene (CT376), and the other ORF shares homology to the glycogen hydrolase gene (CT042). Clone 11-H3-68, (SEQ ID NO: 60), identified using the TCT-3 cell line, has two ORFs with a total insert size of 1180 bp. One partial ORF encodes the plasmid-encoded PGP6-D virulence protein while the second ORF is a complete ORF for the L1 ribosomal gene (CT318). Clone 11-H4-28, (SEQ ID NO: 59), identified using the TCT-3 cell line, has an insert size of 552 bp and is part of the ORF for the dnaK gene (CT396). Clone 12-B3-95, (SEQ ID NO: 58), identified using the TCT-1 cell line, has an insert size of 463 bp and is a part of the ORF for for the lipoamide dehydrogenase gene (CT557). Clones 15-G1-89 and 12-B3-95 are identical, (SEQ ID NO: 55 and 58, respectively), identified using the TCT-1 cell line, has an insert size of 463 bp and is part of the ORF for the lipoamide dehydrogenase gene (CT557). Clone 12-G3-83, (SEQ ID NO: 57), identified using the TCT-1 cell line, has an insert size of 1537 bp and has part of the ORF for the hypothetical protein CT622.

Clone 23-G7-68, (SEQ ID NO: 79), identified using the TCT-3 cell line, contains a 950 bp insert and contains a small part of the L11 ribosomal ORF, the entire ORF for L1 ribosomal protein and a part of the ORF for L10 ribosomal protein. Clone

22-F8-91, (SEQ ID NO: 80), identified using the TCT-1 cell line, contains a 395 bp insert that contains a part of the pmpC ORF on the complementary strand of the clone. Clone 21-E8-95, (SEQ ID NO: 81), identified using the TCT-3 cell line, contains a 2,085 bp insert which contains part of CT613 ORF, the complete ORF for CT612, the complete ORF for CT611 and part of the ORF for CT610. Clone 19-F12-57, (SEQ ID NO: 82), identified using the TCT-3 cell line, contains a 405 bp insert which contains part of the CT 858 ORF and a small part of the recA ORF. Clone 19-F12-53, (SEQ ID NO: 83), identified using the TCT-3 cell line, contains a 379 bp insert that is part of the ORF for CT455 encoding glutamyl tRNA synthetase. Clone 19-A5-54, (SEQ ID NO: 84), identified using the TCT-3 cell line, contains a 715 bp insert that is part of the 10 ORF3 (complementary strand of the clone) of the cryptic plasmid. Clone 17-E11-72, (SEQ ID NO: 85), identified using the TCT-1 cell line, contains a 476 bp insert that is part of the ORF for Opp_2 and pmpD. The pmpD region of this clone is covered by the pmpD region of clone 15-H2-76. Clone 17-C1-77, (SEQ ID NO: 86), identified using the TCT-3 cell line, contains a 1551 bp insert that is part of the CT857 ORF, as well as 15 part of the CT858 ORF. Clone 15-H2-76, (SEQ ID NO: 87), identified using the TCT-1 cell line, contains a 3,031 bp insert that contains a large part of the pmpD ORF, part of the CT089 ORF, as well as part of the ORF for SycE. Clone 15-A3-26, (SEQ ID NO: 88), contains a 976 bp insert that contains part of the ORF for CT858. Clone 17-G4-36, (SEQ ID NO: 267), identified using the TCT-10 cell line, contains a 680 bp insert that 20 is in frame with beta-gal in the plasmid and shares homology to part of the ORF for DNA-directed RNA polymerase beta subunit (CT315 in SerD).

Several of the clones described above share homology to various polymorphic membrane proteins. The genomic sequence of *Chlamydia trachomatis* contains a family of nine polymorphic membrane protein genes, referred to as pmp. These genes are designated pmpA, pmpB, pmpC, pmpD, pmpE, pmpF, pmpG, pmpH and pmpI. Proteins expressed from these genes are believed to be of biological relevance in generating a protective immune response to a *Chlamydial* infection. In particular, pmpC, pmpD, pmpE and pmpI contain predictable signal peptides, suggesting they are outer membrane proteins, and therefore, potential immunological targets.

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Based on the *Chlamydia trachomatis* LGVII serovar sequence, primer pairs were designed to PCR amplify the full-length fragments of pmpC, pmpD, pmpE, pmpG, pmpH and pmpI. The resulting fragments were subcloned into the DNA vaccine vector JA4304 or JAL, which is JA4304 with a modified linker (SmithKline Beecham, London, England). Specifically, PmpC was subcloned into the JAL vector using the 5'

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oligo GAT AGG CGC GCC GCA ATC ATG AAA TTT ATG TCA GCT ACT GCT G and the 3' oligo CAG AAC GCG TTT AGA ATG TCA TAC GAG CAC CGC A, as provided in SEQ ID NO: 197 and 198, respectively. PCR amplification of the gene under conditions well known in the art and ligation into the 5' ASCI/3' MluI sites of the JAL vector was completed after inserting the short nucleotide sequence GCAATC (SEQ ID NO: 199) upstream of the ATG to create a Kozak-like sequence. The resulting expression vector contained the full-length pmpC gene comprising 5325 nucleotides (SEQ ID NO: 173) containing the hypothetical signal sequence, which encodes a 187 kD protein (SEQ ID NO: 179). The pmpD gene was subcloned into the JA4304 vaccine vector following PCR amplification of the gene using the following oligos: 5' oligo-TGC AAT CAT GAG TTC GCA GAA AGA TAT AAA AAG C (SEQ ID NO: 200) and 3' oligo- CAG AGC TAG CTT AAA AGA TCA ATC GCA ATC CAG TAT TC (SEQ ID NO: 201). The gene was ligated into the a 5' blunted HIII/3' MluI site of the JA4304 vaccine vector using standard techniques well known in the art. The CAATC (SEQ ID NO: 202) was inserted upstream of the ATG to create a Kozak-like sequence. This clone is unique in that the last threonine of the HindIII site is missing due to the blunting procedure, as is the last glycine of the Kozak-like sequence. The insert, a 4593 nucleotide fragment (SEQ ID NO: 172) is the full-length gene for pmpD containing the hypothetical signal sequence, which encodes a 161 kD protein (SEQ ID NO: 178). PmpE was subcloned into the JA4304 vector using the 5' oligo- TGC AAT CAT GAA 20 AAA AGC GTT TTT CTT TTT C (SEQ ID NO: 203), and the 3' oligo- CAG AAC GCG TCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 204). Following PCR amplification, the gene was ligated into the 5' blunted HIII/3' MluI site of JA4304. To facilitate this, a short nucleotide sequence, TGCAATC (SEQ ID NO: 293), was added upstream of the initiation codon for creating a Kozak-like sequence and reconstituting the HindIII site. The insert is the full-length pmpE gene (SEQ ID NO: 171) containing the hypothetical signal sequence. The pmpE gene encodes a 105 kD protein (SEQ ID NO: 177). The pmpG gene was PCR amplified using the 5' oligo- GTG CAA TCA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 205), and the 3' oligo- CAG AAC GCG TTT AGA ACC GGA CTT TAC TTC C (SEQ ID NO: 206) and subcloned into the JA4304 vector. Similar cloning strategies were followed for the pmpI and pmpK genes. In addition, primer pairs were designed to PCR amplify the full-length or overlapping fragments of the pmp genes, which were then subcloned for protein expression in the pET17b vector (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel 35 chromatographic methodology provided by Novagen. Several of the genes encoding

the recombinant proteins, as described below, lack the native signal sequence to facilitate expression of the protein. Full-length protein expression of pmpC was accomplished through expression of two overlapping fragments, representing the amino and carboxy termini. Subcloning of the pmpC-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 187, with the corresponding amino acid sequence provided in SEQ ID NO: 195) used the 5' oligo- CAG ACA TAT GCA TCA CCA TCA CCA TCA CGA GGC GAG CTC GAT CCA AGA TC (SEQ ID NO: 207), and the 3' oligo- CAG AGG TAC CTC AGA TAG CAC TCT CTC CTA TTA AAG TAG G (SEQ ID NO: 208) into the 5' NdeI/3' KPN cloning site of the vector. The carboxy terminus portion of the gene, pmpC-carboxy terminal fragment (SEQ ID NO: 186, with the corresponding amino acid sequence provided in SEQ ID NO: 194), was subcloned into the 5' Nhel/3' KPN cloning site of the expression vector using the following primers: 5' oligo- CAG AGC TAG CAT GCA TCA CCA TCA CCA TCA CGT TAA GAT TGA GAA CTT CTC TGG C (SEQ ID NO: 209), and 3' oligo- CAG AGG TAC CTT AGA ATG TCA TAC GAG CAC CGC AG (SEQ ID NO: 210). PmpD was also expressed as two overlapping proteins. The pmpD-amino terminal portion, which lacks the signal sequence, (SEQ ID NO: 185, with the corresponding amino acid sequence provided in SEQ ID NO: 193) contains the initiating codon of the pET17b and is expressed as a 80 kD protein. For protein expression and purification purposes, a sixhistidine tag follows the initiation codon and is fused at the 28th amino acid (nucleotide 84) of the gene. The following primers were used, 5' oligo, CAG ACA TAT GCA TCA CCA TCA CCA TCA CGG GTT AGC (SEQ ID NO: 211), and the 3' oligo- CAG AGG TAC CTC AGC TCC TCC AGC ACA CTC TCT TC (SEQ ID NO: 212), to splice into the 5' NdeI/3' KPN cloning site of the vector. The pmpD-carboxy terminus portion (SEQ ID NO: 184) was expressed as a 92 kD protein (SEQ ID NO: 192). For expression and subsequent purification, an additional methionine, alanine and serine was included, which represent the initiation codon and the first two amino acids from the pET17b vector. A six-histidine tag downstream of the methionine, alanine and serine is fused at the 691st amino acid (nucleotide 2073) of the gene. The 5' oligo- CAG AGC TAG CCA TCA CCA TCA CGG TGC TAT TTC TTG CTT ACG 30 TGG (SEQ ID NO: 213) and the 3' oligo- CAG AGG TAC TTn AAA AGA TCA ATC GCA ATC CAG TAT TCG (SEQ ID NO: 214) were used to subclone the insert into the 5' NheI/3' KPN cloning site of the expression vector. PmpE was expressed as a 106kD protein (SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ The pmpE insert also lacks the native signal sequence. 35 ID NO: 191). amplification of the gene under conditions well known in the art was performed using

the following oligo primers: 5' oligo- CAG AGG ATC CAC ATC ACC ATC ACC ATC ACG GAC TAG CTA GAG AGG TTC (SEQ ID NO: 215), and the 3' oligo-CAG AGA ATT CCT AGA ATC GCA GAG CAA TTT C (SEQ ID NO: 216), and the amplified insert was ligated into a 5' BamHI/3' EcoRI site of JA4304. The short nucleotide sequence, as provided in SEQ ID NO: 217, was inserted upstream of the initiation codon for creating the Kozak-like sequence and reconstituting the HindIII site. The expressed protein contains the initiation codon and the downstream 21 amino acids from the pET17b expression vector, i.e., MASMTGGQQMGRDSSLVPSSDP (SEQ ID NO: 218). In addition, a six-histidine tag is included upstream of the sequence described above and is fused at the 28th amino acid (nucleotide 84) of the gene, which eliminates the hypothetical signal peptide. The sequences provided in SEQ ID NO: 183 with the corresponding amino acid sequence provided in SEQ ID NO: 191 do not include these additional sequences. The pmpG gene (SEQ ID NO: 182, with the corresponding amino acid sequence provided in SEQ ID No; 190) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo-CAG AGG TAC CGC ATC ACC ATC ACC ATC ACA TGA TTC CTC AAG GAA TTT ACG (SEQ ID NO: 219), and the 3' oligo- CAG AGC GGC CGC TTA GAA CCG GAC TTT ACT TCC (SEQ ID NO: 220), and ligated into the 5' KPN/3' NotI cloning site of the expression vector. The expressed protein contains an additional namely, end. amino the at sequence acid amino 20 MASMTGGQQNGRDSSLVPHHHHHH (SEQ ID NO: 221), which comprises the initiation codon and additional sequence from the pET17b expression vector. The pmpI gene (SEQ ID NO: 181, with the corresponding amino acid sequence provided in SEQ ID No; 189) was PCR amplified under conditions well known in the art using the following oligo primers: 5' oligo- CAG AGC TAG CCA TCA CCA TCA CCA TCA 25 CCT CTT TGG CCA GGA TCC C (SEQ ID NO: 222), and the 3' oligo- CAG AAC TAG TCT AGA ACC TGT AAG TGG TCC (SEQ ID NO: 223), and ligted into the expression vector at the 5' Nhel/3' Spel cloning site. The 95 kD expressed protein contains the initiation codon plus an additional alanine and serine from the pET17b vector at the amino end of the protein. In addition, a six-histidine tag is fused at the 21st amino acid of the gene, which eliminates the hypothetical signal peptide.

Clone 14H1-4, (SEQ ID NO: 56), identified using the TCT-3 cell line, contains a complete ORF for the TSA gene, thiol specific antioxidant – CT603 (the CT603 ORF is a homolog of CPn0778 from *C. pnuemoniae*). The TSA open reading frame in clone 14-H1-4 was amplified such that the expressed protein possess an additional methionine and a 6x histidine tag (amino terminal end). This amplified insert

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was sub-cloned into the Nde/EcoRI sites of the pET17b vector. Upon induction of this clone with IPTG, a 22.6 kDa protein was purified by Ni-NTA agarose affinity chromatography. The determined amino acid sequence for the 195 amino acid ORF of clone 14-H1-4 encoding the TSA gene is provided in SEQ ID NO: 65. Further analysis yielded a full-length clone for the TSA gene, referred to as CTL2-TSA-FL, with the full-length amino acid sequence provided in SEQ ID NO: 92.

Further studies yielded 10 additional clones identified by the TCT-1 and TCT-3 T-cell lines, as described above. The clones identified by the TCT-1 line are: 16-D4-22, 17-C5-19, 18-C5-2, 20-G3-45 and 21-C7-66; clones identified by the TCT-3 cell line are: 17-C10-31, 17-E2-9, 22-A1-49 and 22-B3-53. Clone 21-G12-60 was recognized by both the TCT-1 and TCT-3 T cell lines. Clone 16-D4-22 (SEQ ID NO: 119), identified using the TCT-1 cell line contains a 953 bp insert that contains two genes, parts of open reading frame 3 (ORF3) and ORF4 of the C. trachomatis plasmid for growth within mammalian cells. Clone 17-C5-19 (SEQ ID NO: 118), contains a 951 bp insert that contains part of the ORF for DT431, encoding for clpP_1 protease and part of the ORF for CT430 (diaminopimelate epimerase). Clone 18-C5-2 (SEQ ID NO: 117) is part of the ORF for S1 ribosomal protein with a 446 bp insert that was identified using the TCT-1 cell line. Clone 20-G3-45 (SEQ ID NO: 116), identified by the TCT-1 cell line, contains a 437 bp insert that is part of the pmpB gene (CT413). Clone 21-C7-66 (SEQ ID NO: 115), identified by the TCT-1 line, contains a 995bp insert that encodes part of the dnaK like protein. The insert of this clone does not overlap with the insert of the TCT-3 clone 11-H4-28 (SEQ ID NO: 59), which was Clone 17-C10-31 (SEQ ID NO: 114), shown to be part of the dnaK gene CT396 identified by the TCT-3 cell line, contains a 976 bp insert. This clone contains part of the ORF for CT858, a protease containing IRBP and DHR domains. Clone 17-E2-9 (SEQ ID NO: 113) contains part of ORFs for two genes, CT611 and CT610, that span a 1142 bp insert. Clone 22-A1-49 (SEQ ID NO: 112), identified using the TCT-3 line, also contains two genes in a 698 bp insert. Part of the ORF for CT660 (DNA gyrase{gyrA_2}) is present on the top strand where as the complete ORF for a hypothetical protein CT659 is present on the complementary strand. Clone 22-B3-53 30 (SEO ID NO: 111), identified by the TCT-1 line, has a 267 bp insert that encodes part of the ORF for GroEL (CT110). Clone 21-G12-60 (SEQ ID NO: 110), identified by both the TCT-1 and TCT-3 cell lines contains a 1461 bp insert that contains partial ORFs for hypothetical proteins CT875, CT229 and CT228.

Additional Chlamydia antigens were obtained by screening a genomic expression library of Chlamydia trachomatis (LGV II serovar) in Lambda Screen-1

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vector (Novagen, Madison, WI) with sera pooled from several Chlamydia-infected individuals using techniques well known in the art. The following immuno-reactive clones were identified and the inserts containing Chlamydia genes sequenced: CTL2#1 (SEQ ID NO: 71); CTL2#2 (SEQ ID NO: 70); CTL2#3-5' (SEQ ID NO: 72, a first determined genomic sequence representing the 5' end); CTL2#3-3' (SEQ ID NO: 73, a second determined genomic sequence representing the 3' end); CTL2#4 (SEQ ID NO: 53); CTL2#5 (SEQ ID NO: 69); CTL2#6 (SEQ ID NO: 68); CTL2#7 (SEQ ID NO: 67); CTL2#8b (SEQ ID NO: 54); CTL2#9 (SEQ ID NO: 66); CTL2#10-5' (SEQ ID NO: 74, a first determined genomic sequence representing the 5' end); CTL2#10-3' (SEQ ID NO: 75, a second determined genomic sequence representing the 3' end); CTL2#11-5' (SEQ ID NO: 45, a first determined genomic sequence representing the 5' end); CTL2#11-3' (SEQ ID NO: 44, a second determined genomic sequence representing the 3' end); CTL2#12 (SEQ ID NO: 46); CTL2#16-5' (SEQ ID NO: 47); CTL2#18-5' (SEQ ID NO: 49, a first determined genomic sequence representing the 5' end); CTL2#18-3' (SEQ ID NO: 48, a second determined genomic sequence representing the 3' end); CTL2#19-5' (SEQ ID NO: 76, the determined genomic sequence representing the 5' end); CTL2#21 (SEQ ID NO: 50); CTL2#23 (SEQ ID NO: 51; and CTL2#24 (SEQ ID NO: 52).

Additional Chlamydia trachomatis antigens were identified by These studies used sera pooled from several serological expression cloning. Chlamydia-infected individuals, as described above, but, IgA, and IgM antibodies were used in addition to IgG as a secondary antibody. Clones screened by this method enhance detection of antigens recognized by an early immune response to a Chlamydial infection, that is a mucosal humoral immune response. The following immunoreactive clones were characterized and the inserts containing Chlamydia genes sequenced: CTL2gam-1 (SEQ ID NO: 290), CTL2gam-2 (SEQ ID NO: 289), CTL2gam-5 (SEQ ID 25 NO: 288), CTL2gam-6-3' (SEQ ID NO: 287, a second determined genomic sequence representing the 3' end), CTL2gam-6-5' (SEQ ID NO: 286, a first determined genomic sequence representing the 5' end), CTL2gam-8 (SEQ ID NO: 285), CTL2gam-10 (SEQ ID NO: 284), CTL2gam-13 (SEQ ID NO: 283), CTL2gam-15-3' (SEQ ID NO: 282, a second determined genomic sequence representing the 3' end), CTL2gam-15-5' (SEQ ID NO: 281, a first determined genomic sequence representing the 5' end), CTL2gam-17 (SEQ ID NO: 280), CTL2gam-18 (SEQ ID NO: 279), CTL2gam-21 (SEQ ID NO: 278), CTL2gam-23 (SEQ ID NO: 277), CTL2gam-24 (SEQ ID NO: 276), CTL2gam-26 (SEQ ID NO: 275), CTL2gam-27 (SEQ ID NO: 274), CTL2gam-28 (SEQ ID NO: 273), CTL2gam-30-3' (SEQ ID NO: 272, a second determined genomic sequence

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representing the 3' end) and CTL2gam-30-5' (SEQ ID NO: 271, a first determined genomic sequence representing the 5' end).

EXAMPLE 2

INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y
PRODUCTION BY CHLAMYDIA TRACHOMATIS ANTIGENS

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The ability of recombinant *Chlamydia trachomatis* antigens to induce T cell proliferation and interferon-y production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatograph (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. trachomatis* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 µg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 µg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 µl, 50 µl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ is measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving

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an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

Using the above methodology, recombinant 1B1-66 protein (SEQ ID NO: 5) as well as two synthetic peptides corresponding to amino acid residues 48-67 (SEQ ID NO: 13; referred to as 1-B1-66/48-67) and 58-77 (SEQ ID NO: 14, referred to as 1B1-66/58-77), respectively, of SEQ ID NO: 5, were found to induce a proliferative response and IFN-γ production in a Chlamydia-specific T cell line used to screen a genomic library of *C. trachomatis* LGV II.

Further studies have identified a *C. trachomatis*-specific T-cell epitope in the ribosomal S13 protein. Employing standard epitope mapping techniques well known in the art, two T-cell epitopes in the ribosomal S13 protein (rS13) were identified with a *Chlamydia*-specific T-cell line from donor CL-8 (T-cell line TCL-8 EB/DC). Fig. 8 illustrates that the first peptide, rS13 1-20 (SEQ ID NO: 106), is 100% identical with the corresponding *C. pneumoniae* sequence, explaining the cross-reactivity of the T-cell line to recombinant *C. trachomatis*- and *C. pneumoniae*-rS13. The response to the second peptide rS13 56-75 (SEQ ID NO: 108) is *C. trachomatis*-specific, indicating that the rS13 response in this healthy asymptomatic donor was elicited by exposure to *C. trachomatis* and not to *C. pneumoniae*, or any other microbial infection.

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As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5×10^4 TCP-21 T-cells in the presence of 1 $\times 10^4$ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-21 T-cell line also gave a proliferative response to the homologous C. pneumoniae peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the C. trachomatis peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative

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response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between C. trachomatis and C. pneumoniae.

To further define the epitope described above, an additional T-cell line, TCT-3, was used in epitope mapping experiments. The immunoassays were performed as described above, except that only peptides from *C. trachomatis* were tested. The T-cells gave a proliferative response to two peptides, CT-OMCB #152-171 and CT-OMCB #157-176 (SEQ ID NO: 246 and 247, respectively), thereby defining an additional immunogenic epitope in the cysteine rich outer membrane protein of *C. trachomatis*.

Clone 14H1-4, (SEQ ID NO: 56, with the corresponding full-length amino acid sequence provided in SEQ ID NO: 92), was identified using the TCT-3 cell line in the CD4 T-cell expression cloning system previously described, and was shown to contain a complete ORF for the, thiol specific antioxidant gene (CT603), referred to as TSA. Epitope mapping immunoassays were performed, as described above, to further define the epitope. The TCT-3 T-cells line exhibited a strong proliferative response to the overlapping peptides CT-TSA #96-115, CT-TSA #101-120 and CT-TSA #106-125 (SEQ ID NO: 254-256, respectively) demonstrating an immunoreactive epitope in the thiol specific antioxidant gene of *C. trachomatis* serovar LGVII.

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EXAMPLE 3

PREPARATION OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugating or labeling of the peptide. Cleavage of the peptides from the solid support may be carried mixture: trifluoroacetic following cleavage using the out acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the Following lyophilization of the pure fractions, the peptides may be peptides. characterized using electrospray mass spectrometry and by amino acid analysis.

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EXAMPLE 4

ISOLATION AND CHARACTERIZATION OF DNA SEQUENCES ENCODING CHLAMYDIA ANTIGENS USING RETROVIRAL EXPRESSION VECTOR SYSTEMS

AND SUBSEQUENT IMMUNOLOGICAL ANALYSIS

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A genomic library of Chlamydia trachomatis LGV II was constructed by limited digests using BamHI, BgIII, BstYi and MboI restriction enzymes. The restriction digest fragments were subsequently ligated into the BamHI site of the retroviral vectors pBIB-KS1,2,3. This vector set was modified to contain a Kosak translation initiation site and stop codons in order to allow expression of proteins from short DNA genomic fragments, as shown in Fig. 2. DNA pools of 80 clones were prepared and transfected into the retroviral packaging line Phoenix-Ampho, as described in Pear, W.S., Scott, M.L. and Nolan, G.P., Generation of High Titre, Helperfree Retroviruses by Transient Transfection. Methods in Molecular Medicine: Gene Therapy Protocols, Humana Press, Totowa, NJ, pp. 41-57. The Chlamydia library in retroviral form was then transduced into H2-Ld expressing P815 cells, which were then used as target cells to stimulate an antigen specific T-cell line.

A Chlamydia-specific, murine H2^d restricted CD8+ T-cell line was expanded in culture by repeated rounds of stimulation with irradiated C. trachomatis-infected J774 cells and irradiated syngeneic spleen cells, as described by Starnbach, M., in J. Immunol., 153:5183, 1994. This Chlamydia-specific T-cell line was used to screen the above Chlamydia genomic library expressed by the retrovirally-transduced P815 cells. Positive DNA pools were identified by detection of IFN-γ production using Elispot analysis (see Lalvani et al., J. Experimental Medicine 186:859-865, 1997).

Two positive pools, referred to as 2C7 and 2E10, were identified by IFN-γ Elispot assays. Stable transductants of P815 cells from pool 2C7 were cloned by limiting dilution and individual clones were selected based upon their capacity to elicit IFN-γ production from the *Chlamydia*-specific CTL line. From this screening process, four positive clones were selected, referred to as 2C7-8, 2C7-9, 2C7-19 and 2C7-21. Similarly, the positive pool 2E10 was further screened, resulting in an additional positive clone, which contains three inserts. The three inserts are fragments of the CT016, tRNA syntase and clpX genes (SEQ ID NO: 268-270, respectively).

Transgenic DNA from these four positive 2C7 clones were PCR amplified using pBIB-KS specific primers to selectively amplify the *Chlamydia* DNA insert. Amplified inserts were gel purified and sequenced. One immunoreactive clone, 2C7-8 (SEQ ID NO: 15, with the predicted amino acid sequence provided in SEQ ID

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NO: 32), is a 160 bp fragment with homology to nucleotides 597304-597145 of *Chlamydia trachomatis*, serovar D (NCBI, BLASTN search; SEQ ID NO: 33, with the predicted amino acid sequence provided in SEQ ID NO: 34). The sequence of clone 2C7-8 maps within two putative open reading frames from the region of high homology described immediately above, and in particular, one of these putative open reading frames, consisting of a 298 amino acid fragment (SEQ ID NO: 16, with the predicted amino acid sequence provided in SEQ ID NO: 17), was demonstrated to exhibit immunological activity.

Full-length cloning of the 298 amino acid fragment (referred to as CT529 and/or the Cap1 gene) from serovar L2 was obtained by PCR amplification using 5'-ttttgaagcaggtaggtgaatatg (forward) (SEQ ID NO: 159) and 5'-ttaagaaatttaaaaaatccctta (reverse) (SEQ ID NO: 160) primers, using purified C. trachomatis L2 genomic DNA as template. This PCR product was gel-purified, cloned into pCRBlunt (Invitrogen, Carlsbad, CA) for sequencing, and then subcloned into the EcoRI site of pBIB-KMS, a derivative of pBIB-KS for expression. The Chlamydia pnuemoniae homlogue of CT529 is provided in SEQ ID NO: 291, with the corresponding amino acid sequence provided in SEQ ID NO: 292.

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Full-length DNA encoding various CT529 serovars were amplified by PCR from bacterial lysates containing 10⁵ IFU, essentially as described (Denamur, E., C. Sayada, A. Souriau, J. Orfila, A. Rodolakis and J. Elion. 1991. J. Gen. Microbiol. 137: 2525). The following serovars were amplified as described: Ba (SEQ ID NO: 134, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 135); E (BOUR) and E (MTW447) (SEQ ID NO: 122, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 123); F (NI1) (SEQ ID NO: 128, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 129); G; (SEQ ID NO: 126, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 127); Ia (SEQ ID NO: 124, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 125); L1 (SEQ ID NO: 130, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 131); L3 (SEQ ID NO: 132, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 133); I (SEQ ID NO: 263, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 264); K (SEQ ID NO: 265, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 266); and MoPn (SEQ ID NO: 136, with the corresponding predicted amino acid sequence provided in SEQ ID NO: 137). PCR reactions were performed with Advantage Genomic PCR Kit (Clontech, Palo Alto, CA) using primers specific for serovar L2 DNA (external to the ORF). Primers sequences were 5'- ggtataatatctctctaaattttg (forward-SEQ ID NO: 161) and 5'-agataaaaaaggctgtttc' (reverse-SEQ ID NO: 162) except for MoPn which required 5'-ttttgaagcaggtaggtgaatatg (forward-SEQ ID NO: 163) and 5'-tttacaataagaaaagctaagcactttgt (reverse-SEQ ID NO: 164). PCR amplified DNA was purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) and cloned in pCR2.1 (Invitrogen, Carlsbad, CA) for sequencing.

Sequencing of DNA derived from PCR amplified inserts of immunoreactive clones was done on an automated sequencer (ABI 377) using both a pBIB-KS specific forward primer 5'-cettacacagtcctgctgac (SEQ ID NO: 165) and a reverse primer 3'-gtttccgggccctcacattg (SEQ ID NO: 166). PCRBlunt cloned DNA coding for CT529 serovar L2 and pCR2.1 cloned DNA coding for CT529 serovar Ba, E (BOUR), E (MTW447), F (NI1), G, Ia, K, L1, L3 and MoPn were sequenced using T7 promoter primer and universal M13 forward and M13 reverse primers.

To determine if these two putative open reading frames (SEQ ID NO: 16 and 20) encoded a protein with an associated immunological function, overlapping peptides (17-20 amino acid lengths) spanning the lengths of the two open reading frames were synthesized, as described in Example 3. A standard chromium release assay was utilized to determine the per cent specific lysis of peptide-pulsed H2d restricted target cells. In this assay, aliquots of P815 cells (H2^d) were labeled at 37° C for one hour with 100 μCi of ^{51}Cr in the presence or absence of 1 $\mu g/ml$ of the indicated peptides. Following this incubation, labeled P815 cells were washed to remove excess ⁵¹Cr and peptide, and subsequently plated in duplicate in microculture plates at a concentration of 1,000 cells/well. Effector CTL (Chlamydia-specific CD8 T cells) were Following a 4 hour incubation, added at the indicated effector:target ratios. supernatants were harvested and measured by gamma-counter for release of 51Cr into the supernatant. Two overlapping peptides from the 298 amino acid open reading frame did specifically stimulate the CTL line. The peptides represented in SEQ ID NO: 138-156 were synthesized, representing the translation of the L2 homologue of the serovar D open reading frame for CT529 (Cap1 gene) and 216 amino acid open reading frame. As shown in Fig. 3, peptides CtC7.8-12 (SEQ ID NO: 18, also referred to as Cap1#132-147, SEQ ID NO: 139) and CtC7.8-13 (SEQ ID NO: 19, also referred to as Cap1#138-155, SEQ ID NO: 140) were able to elicit 38 to 52% specific lysis, respectively, at an effector to target ratio of 10:1. Notably, the overlap between these two peptides contained a predicted H2^d (K^d and L^d) binding peptide. A 10 amino acid peptide was synthesized to correspond to this overlapping sequence (SEQ ID NO: 31) and was found to generate a strong immune response from the anti-Chlamydia CTL line by elispot assay. Significantly, a search of the most recent Genbank database revealed no

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proteins have previously been described for this gene. Therefore, the putative open reading frame encoding clone 2C7-8 (SEQ ID NO: 15) defines a gene which encompasses an antigen from *Chlamydia* capable of stimulating antigen-specific CD8+ T-cells in a MHC-I restricted manner, demonstrating this antigen could be used to develop a vaccine against *Chlamydia*.

To confirm these results and to further map the epitope, truncated peptides (SEQ ID NO: 138-156) were made and tested for recognition by the T-cells in an IFN-g ELISPOT assay. Truncations of either Ser139 (Cap1#140-147, SEQ ID NO: 146) or Leu147 (Cap1#138-146, SEQ ID NO: 147) abrogate T-cell recognition. These results indicate that the 9-mer peptide Cap1#139-147 (SFIGGITYL, SEQ ID NO: 145) is the minimal epitope recognized by the *Chlamydia*-specific T-cells.

Sequence alignments of Cap1 (CT529) from selected serovars of *C. trachomatis* (SEQ ID NO: 121, 123, 125, 127, 129, 131, 133, 135, 137 and 139) shows one of the amino acid differences is found in position 2 of the proposed epitope. The homologous serovar D peptide is SIIGGITYL (SEQ ID NO: 168). The ability of SFIGGITYL and SIIGGITYL to target cells for recognition by the *Chlamydia* specific T-cells was compared. Serial dilutions of each peptide were incubated with P815 cells and tested for recognition by the T-cells in a ⁵¹Cr release assay, as described above. The *Chlamydia*-specific T-cells recognize the serovar L2 peptide at a minimum concentration of 1 nM and the serovar D peptide at a minimum concentration of 10 nM.

Further studies have shown that a Cap1#139-147-specific T-cell clone recognizes *C. trachomatis* infected cells. To confirm that Cap1₁₃₉₋₁₄₇ is presented on the surface of *Chlamydia* infected cells, Balb-3T3 (H-2^d) cells were infected with *C. trachomatis* serovar L2 and tested to determine whether these cells are recognized by a CD8+ T-cell clone specific for Cap1#139-147 epitope (SEQ ID NO: 145). The T-cell clone specific for Cap1#139-147 epitope was obtained by limiting dilution of the line 69 T-cells. The T-cell clone specifically recognized the *Chlamydia* infected cells. In these experiments, target cells were *C. trachomatis* infected (positive control) or uninfected Balb/3T3 cells, showing 45%, 36% and 30% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively; or Cap1#139-147 epitope (SEQ ID NO: 145) coated, or untreated P815 cells, showing 83%, 75% and 58% specific lysis at 30:1, 10:1 and 3:1 effector to target ratios, respectively (negative controls having less than 5% lysis in all cases). This data suggests that the epitope is presented during infection.

In vivo studies show Cap1#139-147 epitope-specific T-cells are primed during murine infection with C. trachomatis. To determine if infection with C. trachomatis primes a Cap1#139-147 epitope-specific T-cell response, mice were

infected i.p. with 10⁸ IFU of *C. trachomatis* serovar L2. Two weeks after infection, the mice were sacrificed and spleen cells were stimulated on irradiated syngeneic spleen cells pulsed with Cap1#139-147 epitope peptide. After 5 days of stimulation, the cultures were used in a standard ⁵¹Cr release assay to determine if there were Cap1#139-147 epitope-specific T-cells present in the culture. Specifically, spleen cells from a *C. trachomatis* serovar L2 immunized mouse or a control mouse injected with PBS after a 5 days culture with Cap1#139-147 peptide-coated syngeneic spleen cells and CD8+ T-cells able to specifically recognize Cap1#139-147 epitope gave 73%, 60% and 32% specific lysis at a30:1, 10:1 and 3:1 effector to target ratios, respectively. The control mice had a percent lysis of approximately 10% at a 30:1 effector to target ratio, and steadily declining with lowering E:T ratios. Target cells were Cap1#139-147 peptide-coated, or untreated P815 cells. These data suggest that Cap1#139-147 peptide-specific T-cells are primed during murine infection with *C. trachomatis*.

Studies were performed demonstrating that Ct529 (referred to herein as Cap-1) localizes to the inclusion membrane of C. trachomatis-infected cells and is not 15 associated with elementary bodies or reticulate bodies. As described above, Cap-1 was identified as a product from Chlamydia that stimulates CD8+ CTL. These CTL are protective in a murine model of infection, thus making Cap-1 a good vaccine candidate. Further, since these CTL are MHC-I restricted, the Cap-1 gene must have access to the cytosol of infected cells, which may be a unique characteristic of specific Chlamydial Therefore, determination of the cellular localization of the gene 20 gene products. products would be useful in characterizing Cap-1 as a vaccine candidate. To detect the intracellular localization of Cap-1, rabbit polyclonal antibodies directed against a recombinant polypeptide encompassing the N-terminal 125 amino acids of Cap-1 (SEQ ID NO: 305, with the amino acid sequence including the N-terminal 6-His tag provided in SEQ ID NO: 304) were used to stain McCoy cells infected with Chlamydiae.

Rabbit-anti-Cap-1 polyclonal antibodies were obtained by hyper-immunization of rabbits with a recombinant polypeptide, rCt529c1-125 (SEQ ID NO: 305) encompassing the N-terminal portion of Cap-1. Recombinant rCt529e1-125 protein was obtained from *E. coli* transformed with a pET expression plasmid (as described above) encoding the nucleotides 1-375 encoding the N-terminal 1-125 amino acids of Cap-1. Recombinant protein was purified by Ni-NTA using techniques well known in the art. For a positive control antiserum, polyclonal antisera directed against elementary bodies were made by immunization of rabbits with purified *C. trachomatis* elementary bodies (Biodesign, Sacco, Maine). Pre-immune sera derived from rabbits prior to immunization with the Cap-1 polypeptide was used as a negative control.

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Immunocytochemistry was performed on McCoy cell monolayers grown on glass coverslips inoculated with either *C. trachomatis* serovar L2 or *C. psitacci*, strain 6BC, at a concentration of 10⁶ IFU (Inclusion Forming Units) per ml. After 2 hours, medium was aspirated and replaced with fresh RP-10 medium supplemented with cycloheximide (1.0 µg/ml). Infected cells were incubated at in 7% CO₂ for 24 hours and fixed by aspirating medium, rinsing cells once with PBS and methanol fixation for 5 minutes. For antigen staining, fixed cell monolayers were washed with PBS and incubated at 37°C for 2 hours with 1:100 dilutions of specific or control antisera. Cells were rinsed with PBS and incubated for 1 hour with fluorescein isothiocyanate (FITC)-labeled, anti-rabbit IgG (KPL, Gaithersburg) and stained with Evans blue (0.05%) in PBS. Fluorescence was observed with a 100X objective (Zeiss epifluorescence microscope), and photographed (Nikon UFX-11A camera).

Results from this study show Cap-1 localizes to the inclusion membrane of *C. trachomatis*-infected cells. Cap-1 specific antibody labeled the inclusion membranes of *C. trachomatis*-infected cells, but not *Chlamydial* elementary bodies contained in these inclusions or released by the fixation process. Conversely, the anti-elementary body antibody clearly labeled the bacterial bodies, not only within the inclusions, but those released by the fixation process. Specificity of the anti-Cap-1 antibody is demonstrated by the fact that it does not stain *C. psittaci*-infected cells. Specificity of the Cap-1 labeling is also shown by the absence of reactivity in pre-immune sera. These results suggest that Cap-1 is released from the bacteria and becomes associated with the *Chlamydial* inclusion membrane. Therefore, Cap-1 is a gene product which may be useful for stimulating CD8+ T cells in the development of a vaccine against infections caused by *Chlamydia*.

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The relevance of the Cap-1 gene as a potential CTL antigen in a vaccine against *Chlamydia* infection is further illustrated by two additional series of studies. First, CTL specific for the MHC-I epitope of Cap-1 CT529 #138-147 peptide of *C. trachomatis* (SEQ ID NO: 144) have been shown to be primed to a high frequency during natural infection. Specifically, Balb/C mice were inoculated with 10⁶ I.F.U. of *C. trachomatis*, serova L2. After 2 weeks, spleens were harvested and quantified by Elispot analysis for the number of IFN-γ secreting cells in response to Cap-1 #138-147 peptide-pulsed antigen presenting cells. In two experiments, the number of IFN-γ-secreting cells in 10⁵ splenocytes was about 1% of all CD8+ T-cells. This high frequency of responding CD8+ CTL to the MHC-1 epitope (Cap-1 CT529 #138-147 peptide) suggest that Cap-1 is highly immunogenic in infections.

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Results from a second series of studies have shown that the Cap-1 protein is almost immediately accessible to the cytosol of the host cell upon infection. This is shown in a time-course of Cap-1 CT529 #138-147 peptide presentation. Briefly, 3T3 cells were infected with *C. trachomatis* serovar L2 for various lengths of time, and then tested for recognition by Cap-1 CT529 #138-147 peptide-specific CTL. The results show that *C. trachomatis*-infected 3T3 cells are targeted for recognition by the antigen-specific CTL after only 2 hours of infection. These results suggest that Cap-1 is an early protein synthesized in the development of *C. trachomatis* elementary bodies to reticulate bodies. A CD8+ CTL immune response directed against a gene product expressed early in infection may be particularly efficacious in a vaccine against *Chlamydia* infection.

EXAMPLE 5

GENERATION OF ANTIBODY AND T-CELL RESPONSES IN MICE IMMUNIZED WITH CHLAMYDIA ANTIGENS

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Immunogenicity studies were conducted to determine the antibody and CD4+ T cell responses in mice immunized with either purified SWIB or S13 proteins formulated with Montanide adjuvant, or DNA-based immunizations with pcDNA-3 expression vectors containing the DNA sequences for SWIB or S13. SWIB is also referred to as clone 1-B1-66 (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5), and S13 ribosomal protein is also referred to as clone 10-C10-31 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12). In the first experiment, groups of three C57BL/6 mice were immunized twice and monitored for antibody and CD4+ T-cell responses. immunizations were intradermal at the base of the tail and polypeptide immunizations were administered by subcutaneous route. Results from standard ³H-incorporation assays of spleen cells from immunized mice shows a strong proliferative response from the group immunized with purified recombinant SWIB polypeptide (SEQ ID NO: 5). Further analysis by cytokine induction assays, as previously described, demonstrated that the group immunized with SWIB polypeptide produced a measurable IFN-y and IL-4 response. Subsequent ELISA-based assays to determine the predominant antibody isotype response in the experimental group immunized with the SWIB polypeptide were performed. Fig. 4 illustrates the SWIB-immunized group gave a humoral response that was predominantly IgG1.

In a second experiment, C3H mice were immunized three times with 10 µg purified SWIB protein (also referred to as clone 1-B1-66, SEQ ID NO: 5)

formulated in either PBS or Montanide at three week intervals and harvested two weeks after the third immunization. Antibody titers directed against the SWIB protein were determined by standard ELISA-based techniques well known in the art, demonstrating the SWIB protein formulated with Montanide adjuvant induced a strong humoral immune response. T-cell proliferative responses were determined by a XTT-based assay (Scudiero, et al, *Cancer Research*, 1988, 48:4827). As shown in Fig. 5, splenocytes from mice immunized with the SWIB polypeptide plus Montanide elicited an antigen specific proliferative response. In addition, the capacity of splenocytes from immunized animals to secrete IFN-γ in response to soluble recombinant SWIB polypeptide was determined using the cytokine induction assay previously described. The splenocytes from all animals in the group immunized with SWIB polypeptide formulated with montanide adjuvant secreted IFN-γ in response to exposure to the SWIB Chlamydia antigen, demonstrating an *Chlamydia*-specific immune response.

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In a further experiment, C3H mice were immunized at three separate time points at the base of the tail with 10 µg of purified SWIB or S13 protein (C. 15 trachomatis, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) formulated with the SBAS2 adjuvant (SmithKline Beecham, London, England). Antigen-specific antibody titers were measured by ELISA, showing both polypeptides induced a strong IgG response, ranging in titers from 1 x10⁻⁴ to 1 x10⁻⁵ 5. The IgG1 and IgG2a components of this response were present in fairly equal 20 amounts. Antigen-specific T-cell proliferative responses, determined by standard ³Hincorporation assays on spleen cells isolated from immunized mice, were quite strong for SWIB (50,000 cpm above the negative control) and even stronger for s13 (100,000 cpm above the negative control). The IFNy production was assayed by standard ELISA techniques from supernatant from the proliferating culture. In vitro restimulation of the 25 culture with S13 protein induced high levels of IFNy production, approximately 25 ng/ml versus 2 ng/ml for the negative control. Restimulation with the SWIB protein also induced IFNy, although to a lesser extent.

In a related experiment, C3H mice were immunized at three separate time points with 10 μg of purified SWIB or S13 protein (*C. trachomatis*, SWIB protein, clone 1-B1-66, SEQ ID NO: 5, and S13 protein, clone 10-C10-31, SEQ ID NO: 4) mixed with 10 μg of Cholera Toxin. Mucosal immunization was through intranasal inoculation. Antigen-specific antibody responses were determined by standard ELISA techniques. Antigen-specific IgG antibodies were present in the blood of SWIB-immunized mice, with titers ranging from 1 x10⁻³ to 1 x10⁻⁴, but non-detectable in the S13-immunized animals. Antigen-specific T-cell responses from isolated splenocytes,

as measured by IFNy production, gave similar results to those described immediately above for systemic immunization.

An animal study was conducted to determine the immunogenicity of the CT529 serovar LGVII CTL epitope, defined by the CT529 10mer consensus peptide (CSFIGGITYL - SEQ ID NO: 31), which was identified as an H2-Kd restricted CTL epitope. BALB/c mice (3 mice per group) were immunized three times with 25 µg of peptide combined with various adjuvants. The peptide was administered systemically at the base of the tail in either SKB Adjuvant System SBAS-2", SBAS-7 (SmithKline Beecham, London, England) or Montanide. The peptide was also administered intranasally mixed with 10ug of Cholera Toxin (CT). Naive mice were used as a control. Four weeks after the 3rd immunization, spleen cells were restimulated with LPS-blasts pulsed with 10ug/ml CT529 10mer consensus peptide at three different effector to LPS-blasts ratios: 6, 1.5 and 0.4 at 1x106 cell/ml. After 2 restimulations, effector cells were tested for their ability to lyse peptide pulsed P815 cells using a standard chromium release assay. A non-relevant peptide from chicken egg ovalbumin was used as a negative control. The results demonstrate that a significant immune response was elicited towards the CT529 10mer consensus peptide and that antigenspecific T-cells capable of lysing peptide-pulsed targets were elicited in response to immunization with the peptide. Specifically, antigen-specific lytic activities were found in the SBAS-7 and CT adjuvanted group while Montanide and SBAS-2" failed to adjuvant the CTL epitope immunization.

EXAMPLE 6

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EXPRESSION AND CHARACTERIZATION OF CHLAMYDIA PNEUMONIAE GENES

The human T-cell line, TCL-8, described in Example 1, recognizes Chlamydia trachomatis as well as Chlamydia pneumonia infected monocyte-derived dendritic cells, suggesting Chlamydia trachomatis and pneumonia may encode cross-reactive T-cell epitopes. To isolate the Chlamydia pneumonia genes homologous to Chlamydia trachomatis LGV II clones 1B1-66, also referred to as SWIB (SEQ ID NO: 1) and clone 10C10-31, also referred to as S13 ribosomal protein (SEQ ID NO: 4), HeLa 229 cells were infected with C. pneumonia strain TWAR (CDC/CWL-029). After three days incubation, the C. pneumonia-infected HeLa cells were harvested, washed and resuspended in 200 µl water and heated in a boiling water bath for 20 minutes. Ten microliters of the disrupted cell suspension was used as the PCR template.

C. pneumonia specific primers were designed for clones 1B1-66 and 10C10-31 such that the 5' end had a 6X-Histidine tag and a Nde I site inserted, and the

3' end had a stop codon and a BamHI site included (Fig. 6). The PCR products were amplified and sequenced by standard techniques well known in the art. The *C. pneumonia*-specific PCR products were cloned into expression vector pET17B (Novagen, Madison, WI) and transfected into E. coli BL21 pLysS for expression and subsequent purification utilizing the histidine-nickel chromatographic methodology provided by Novagen. Two proteins from *C. pneumonia* were thus generated, a 10-11 kDa protein referred to as CpSWIB (SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively), a 15 kDa protein referred to as CpS13 (SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively).

EXAMPLE 7

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INDUCTION OF T CELL PROLIFERATION AND INTERFERON-Y PRODUCTION BY CHLAMYDIA PNEUMONIAE ANTIGENS

The ability of recombinant *Chlamydia pneumoniae* antigens to induce T cell proliferation and interferon-γ production is determined as follows.

Proteins are induced by IPTG and purified by Ni-NTA agarose affinity chromatography (Webb et al., *J. Immunology 157*:5034-5041, 1996). The purified polypeptides are then screened for the ability to induce T-cell proliferation in PBMC preparations. PBMCs from *C. pneumoniae* patients as well as from normal donors whose T-cells are known to proliferate in response to *Chlamydia* antigens, are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides are added in duplicate at concentrations of 0.5 to 10 μg/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium is removed from each well for determination of IFN-γ levels, as described below. The plates are then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates are coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells are then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates are washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates are incubated overnight at

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room temperature. The plates are again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum is added to each well. The plates are then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) is added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates are washed and TMB substrate added. The reaction is stopped after 20 min with 1 N sulfuric acid. Optical density is determined at 450 nm using 570 nm as a reference wavelength. Fractions that result in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, are considered positive.

A human anti-Chlamydia T-cell line (TCL-8) capable of cross-reacting to C. trachomatis and C. pneumonia was used to determine whether the expressed proteins described in the example above, (i.e., CpSWIB, SEQ ID NO: 27, and SEQ ID NO: 78 having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 28, respectively, and the 15 kDa protein referred to as CpS13 SEQ ID NO: 29, and SEQ ID NO: 77, having a 6X His tag, with the corresponding amino acid sequence provided in SEQ ID NO: 30 and 91, respectively), possessed T-cell epitopes common to both C. trachomatis and C. pneumonia. Briefly, E. coli expressing Chlamydial proteins were titered on 1 x 104 monocyte-derived dendritic cells. After two hours, the dendritic cells cultures were washed and 2.5 x 104 T cells (TCL-8) added and allowed to incubate for an additional 72 hours. The amount of INF-γ in the culture supernatant was then determined by ELISA. As shown in Figs. 7A and 7B, the TCL-8 T-cell line specifically recognized the S13 ribosomal protein from both C. trachomatis and C. pneumonia as demonstrated by the antigen-specific induction of IFN-γ, whereas only the SWIB protein from C. trachomatis was recognized by the T-cell line. To validate these results, the T cell epitope of C. trachomatis SWIB was identified by epitope mapping using target cells pulsed with a series of overlapping peptides and the T-cell line TCL-8. 3H-thymidine incorporation assays demonstrated that the peptide, referred to as C.t.SWIB 52-67, of SEQ ID NO: 39 gave the strongest proliferation of the TCL-8 line. The homologous peptides corresponding to the SWIB of C. pneumoniae sequence (SEQ ID NO: 40), the topoisomerase-SWIB fusion of C. pneumoniae (SEQ ID NO: 43) and C. trachomatis (SEQ ID NO: 42) as well as the human SWI domain (SEQ ID NO: 41) were synthesized and tested in the above assay. The T-cell line TCL-8 only recognized the C. trachomatis peptide of SEQ ID NO: 39 and not the corresponding C. pneumoniae peptide (SEQ ID NO: 40), or the other corresponding 35 peptides described above (SEQ ID NO; 41-43).

Chlamydia-specific T cell lines were generated from donor CP-21 with a positive serum titer against *C. pneumoniae* by stimulating donor PBMC with either *C. trachomatis* or *C. pneumoniae*-infected monocyte-derived dendritic cells, respectively. T-cells generated against *C. pneumoniae* responded to recombinant *C. pneumoniae*-SWIB but not *C. trachomatis*-SWIB, whereas the T-cell line generated against *C. trachomatis* did not respond to either *C. trachomatis*- or *C. pneumoniae*-SWIB (see Fig. 9). The *C. pneumoniae*-SWIB specific immune response of donor CP-21 confirms the *C. pneumoniae* infection and indicates the elicitation of *C. pneumoniae*-SWIB specific T-cells during *in vivo C. pneumoniae* infection.

Epitope mapping of the T-cell response to *C. pneumoniae*-SWIB has shown that Cp-SWIB-specific T-cells responded to the overlapping peptides Cp-SWIB 32-51 (SEQ ID NO: 101) and Cp-SWIB 37-56 (SEQ ID NO: 102), indicating a *C. pneumoniae*-SWIB-specific T-cell epitope Cp-SWIB 37-51 (SEQ ID NO: 100).

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In additional experiments, T-cell lines were generated from donor CP1, also a C. pneumoniae seropositive donor, by stimulating PBMC with non-infectious elementary bodies from C. trachomatis and C. pneumoniae, respectively. In particular, proliferative responses were determined by stimulating 2.5 x 10⁴ T-cells in the presence of 1 x 104 monocyte-derived dendritic cells and non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or either recombinant C. trachomatis or C. pneumoniae SWIB protein. The T-cell response against SWIB resembled the data 20 obtained with T-cell lines from CP-21 in that C. pneumoniae-SWIB, but not C. trachomatis-SWIB elicited a response by the C. pneumoniae T-cell line. In addition, the C. trachomatis T-cell line did not proliferate in response to either C. trachomatis or C. pneumoniae SWIB, though it did proliferate in response to both CT and CP elementary bodies. As described in Example 1, Clone 11-C12-91 (SEQ ID NO: 63), identified using the TCP-21 cell line, has a 269 bp insert that is part of the OMP2 gene (CT443) and shares homology with the 60 kDa cysteine rich outer membrane protein of C. pneumoniae, referred to as OMCB. To further define the reactive epitope(s), epitope mapping was performed using a series of overlapping peptides and the immunoassay previously described. Briefly, proliferative responses were determined by stimulating 2.5 x 10⁴ TCP-21 T-cells in the presence of 1 x 10⁴ monocyte-derived dendritic cells with either non-infectious elementary bodies derived from C. trachomatis and C. pneumoniae, or peptides derived from the protein sequence of C. trachomatis or C. pneumoniae OMCB protein (0.1 µg/ml). The TCP-21 T-cells responded to epitopes CT-OMCB #167-186, CT-OMCB #171-190, CT-OMCB #171-186, and to a lesser extent, CT-OMCB #175-186 (SEQ ID NO: 249-252, respectively). Notably, the TCP-

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21 T-cell line also gave a proliferative response to the homologous *C. pneumoniae* peptide CP-OMCB #171-186 (SEQ ID NO: 253), which was equal to or greater than the response to the to the *C. trachomatis* peptides. The amino acid substitutions in position two (i.e., Asp for Glu) and position four (i.e., Cys for Ser) did not alter the proliferative response of the T-cells and therefore demonstrating this epitope to be a cross-reactive epitope between *C. trachomatis* and *C. pneumoniae*.

EXAMPLE 8

IMMUNE RESPONSES OF HUMAN PBMC AND T-CELL LINES AGAINST CHLAMYDIA ANTIGENS

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The examples provided herein suggest that there is a population of healthy donors among the general population that have been infected with C. trachomatis and generated a protective immune response controlling the C. trachomatis infection. These donors remained clinically asymptomatic and seronegative for C. To characterize the immune responses of normal donors against trachomatis. chlamydial antigens which had been identified by CD4 expression cloning, PBMC obtained from 12 healthy donors were tested against a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and C. trachomatis-, C. pneumoniae-S13. The data are summarized in Table I below. All donors were seronegative for C. trachomatis, whereas 6/12 had a positive C. pneumoniae titer. Using a stimulation index of >4 as a positive response, 11/12 of the subjects responded to C. trachomatis elementary bodies and 12/12 responded to C. pneumoniae elementary bodies. One donor, AD104, responded to recombinant C. pneumoniae-S13 protein, but not to recombinant C. trachomatis-S13 protein, indicating a C. pneumoniae-specific Three out of 12 donors had a C. trachomatis-SWIB, but not a C. response. pneumoniae-SWIB specific response, confirming a C. trachomatis infection. C. trachomatis and C. pneumoniae-S13 elicited a response in 8/12 donors suggesting a chlamydial infection. These data demonstrate the ability of SWIB and S13 to elicit a Tcell response in PBMC of normal study subjects.

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Immune response of normal study subjects against Chlamydia

Donor	Sex	Chlamydia IgG titer	CT EB	CP EB	CT Swib	CP Swib	CT S13	CP S13	CT lpdA	CT TSA
AD100	male	negative	++	+++	+		++	++	-	n.t.
AD104	female	negative	+++	++	-	_	-	++		n.t.
AD108	male	CP 1:256	++	++	+	+/-	+	+	. +	n.t.
AD112	female	negative	++	++	+	-	+	-	+/-	n.t.
AD120	male	negative	-	+	-	-	-	-	-	n.t.
AD124	female	CP 1:128	++	++	-	-	-	-	-	n.t.
AD128	male	CP 1:512	+	++	-	-	++	+	++	-
AD132	female	negative	++	++	-	-	+	+	-	-
AD136	female	CP 1:128	+	++	-	-	+/-	-	-	-
AD140	male	CP 1:256	++	++	-	-	+	+	-	-
AD142	female	CP 1:512	++	++	•	-	+	+	+	-
AD146	female	negative	++	++			++	+	+	

CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary 5 bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein. Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10⁵ PBMC with 1 x 10⁴ monocyte-derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a 3Hthymidine pulse for the last 18h.

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	SI: Sti	mulatio	n index
	+/-:	SI ~	4
	+:	SI>	4
	++:	SI	10-30
5	+++•	SI>	. 30

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In a first series of experiments, T-cell lines were generated from a healthy female individual (CT-10) with a history of genital exposure to *C. trachomatis* by stimulating T-cells with *C. trachomatis* LGV II elementary bodies as previously described. Although the study subject was exposed to *C. trachomatis*, she did not seroconvert and did not develop clinical symptoms, suggesting donor CT-10 may have developed a protective immune response against *C. trachomatis*. As shown in Fig. 10, a primary *Chlamydia*-specific T-cell line derived from donor CT-10 responded to *C. trachomatis*-SWIB, but not *C. pneumoniae*-SWIB recombinant proteins, confirming the exposure of CT-10 to *C. trachomatis*. Epitope mapping of the T-cell response to *C. trachomatis*-SWIB showed that this donor responded to the same epitope Ct-SWIB 52-67 (SEQ ID NO: 39) as T-cell line TCL-8, as shown in Fig. 11.

Additional T-cell lines were generated as described above for various C. trachomatis patients. A summary of the patients' clinical profile and proliferative responses to various C. trachomatis and C. pneumoniae elementary bodies and recombinant proteins are summarized in Table II.

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TABLE II

Proliferative response of C. trachomatis patients										
Patients	Clinical manifestation	IgG titer	CT EB	CP EB	CT Swib	CP Swib	CT S13	CP S13	CT IpdA	CT TSA
CT-1	NGU	negative	+	+		-	++	++	++	+
CT-2	NGU	negative	++	++	-	-	+	+/-	_	-
CT-3	asymptomatic shed Eb Dx was HPV	Ct 1:512 Cp 1:1024 Cps 1:256	+	+	-	-	+	-	+	-
CT-4	asymptomatic shed Eb	Ct 1:1024	+	+	-	-	-	-	-	-
CT-5	\mathbf{BV}	Ct 1:256 Cp 1:256	++	++	-	-	+	-	-	-
CT-6	perinial rash discharge	Cp 1:1024	+	+	-	-	-	-	-	-
CT-7	BV genital ulcer	Ct 1:512 Cp 1:1024	+	+	-	-	+	+	+	-
CT-8	Not known	Not tested	++	++	-	-	-	-	-	-
CT-9	asymptomatic	Ct 1:128 Cp 1:128	+++	++	-	- ·	++	+	. +	-
CT-10	Itch mild vulvar	negative	++	++	-	-	-	-	-	-
CT-11	BV, abnormal pap	Ct 1: 512	+++	+++	-	-	+++	+/-	++	+
CT-12	asymptomatic	Cp 1: 512	++	++	-	-	++	+	+	-

NGU= Non-Gonococcal Urethritis; BV= Bacterial Vaginosis; CT= Chlamydia trachomatis; CP= Chlamydia pneumoniae; EB= Chlamydia elementary bodies; Swib= recombinant Chlamydia Swib protein; S13= recombinant Chlamydia S13 protein; lpdA= recombinant Chlamydia lpdA protein; TSA= recombinant Chlamydia TSA protein

Values represent results from standard proliferation assays. Proliferative responses were determined by stimulating 3 x 10^5 PBMC with 1 x 10^4 monocyte-

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derived dendritic cells pre-incubated with the respective recombinant antigens or elementary bodies (EB). Assays were harvested after 6 days with a ³H-thymidine pulse for the last 18 hours.

5 SI: Stimulation index

+/-:	SI ~	4
+:	SI>	4
++:	SI	10-30
+++:	SI>	30

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Using the panel of asymptomatic (as defined above) study subjects and C. trachomatis patients, as summarized in Tables I and II, a comprehensive study of the immune responses of PBMC derived from the two groups was conducted. Briefly, PBMCs from C. pneumoniae patients as well as from normal donors are cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μ g/ml gentamicin. Purified polypeptides, a panel of recombinant chlamydial antigens including C. trachomatis-, C. pneumoniae-SWIB and S13, as well as . C. trachomatis lpdA and TSA are added in duplicate at concentrations of 0.5 to 10 μ g/mL. After six days of culture in 96-well round-bottom plates in a volume of 200 μ l, 50 μ l of medium is removed from each well for determination of IFN- γ levels, as described below. The plates are then pulsed with 1 μ Ci/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that result in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone are considered positive.

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Proliferative responses to the recombinant *Chlamydiae* antigens demonstrated that the majority of asymptomatic donors and *C. trachomatis* patients recognized the *C. trachomatis* S13 antigen (8/12) and a majority of the *C. trachomatis* patients recognized the *C. pneumonia* S13 antigen (8/12), with 4/12 asymptomatic donors also recognizing the *C. pneumonia* S13 antigen. Also, six out of twelve of the *C. trachomatis* patients and four out of twelve of the asymptomatic donors gave a proliferative response to the lpdA antigen of *C. trachomatis*. These results demonstrate that the *C. trachomatis* and *C. pneumonia* S13 antigen, *C. trachomatis* Swib antigen and the *C. trachomatis* lpdA antigen are recognized by the asymptomatic donors, indicating these antigens were recognized during exposure to *Chlamydia* and an immune response elicited against them. This implies these antigens may play a role in conferring protective immunity in a human host. In addition, the *C. trachomatis* patients, pneumonia S13 antigen is recognized equally well among the *C. trachomatis* patients,

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therefore indicating there may be epitopes shared between *C. trachomatis* and *C. pneumonia* in the S13 protein. Table III summarizes the results of these studies.

TABLE III

	NORMAL DONORS	C.T. PATIENTS
A. Antigen		
C.tSwib	3/12	0/12
C.pSwib	0/12	0/12
C.tS13	8/12	8/12
C.pS13	4/12	8/12
lpdA	4/12	6/12
TSA	0/12	2/12

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A series of studies were initiated to determine the cellular immune response to short-term T-cell lines generated from asymptomatic donors and C. Cellular immune responses were measured by standard trachomatis patients. proliferation assays and IFN-7, as described in Example 7. Specifically, the majority of the antigens were in the form of single E. coli clones expressing Chlamydial antigens, although some recombinant proteins were also used in the assays. The single E. coli clones were titered on 1 x 104 monocyte-derived dendritic cells and after two hours, the culture was washed and 2.5 x 10⁴ T-cells were added. The assay using the recombinant proteins were performed as previously described. Proliferation was determined after four days with a standard ³H-thymidine pulse for the last 18 hours. Induction of IFN-γ was determined from culture supernatants harvested after four days using standard ELISA assays, as described above. The results show that all the C. trachomatis antigens tested, except for C.T. Swib, elicited a proliferative response from one or more different T-cell lines derived form C. trachomatis patients. In addition, proliferative responses were elicited from both the C. trachomatis patients and asymptomatic donors for the following Chlamydia genes, CT622, groEL, pmpD, CT610 and rS13.

The 12G3-83 clone also contains sequences to CT734 and CT764 in addition to CT622, and therefore these gene sequence may also have immunoreactive epitopes. Similarly, clone 21G12-60 contains sequences to the hypothetical protein genes CT229 and CT228 in addition to CT875; and 15H2-76 also contains sequences

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from CT812 and CT088, as well as sharing homology to the sycE gene. Clone 11H3-61 also contains sequences sharing homology to the PGP6-D virulence protein.

TABLE IV

Clone	C. t. Antigen (putative*)	TCL from Asymp. Donors	TCL from C. t. Patients	SEQ ID NO:
1B1-66 (E. coli)	Swib	2/2	0/4	5
1B1-66 (protein)	Swib	2/2	0/4	5
12G3-83 (E. coli)	CT622*	2/2	4/4	57
22B3-53 (E. coli)	GROEL	1/2	4/4	111
22B3-53 (protein)	GROEL	1/2	4/4	111
15H2-76 (E. coli)	PMPD*	1/2	3/4	87
11H3-61 (E. coli)	rL1*	0/2	3/4	60
14H1-4 (E. coli)	TSA	0/2	3/4	56
14H1-4 (protein)	TSA	0/2	3/4	56
11G10-46 (E. coli)	CT610	1/2	1/4	62
10C10-17 (E. coli)	rS13	1/2	1/4	62
10C10-17 (protein)	RS13	1/2	1/4	62
21G12-60 (E. coli)	CT875*	0/2	2/4	110
11H4-32 (E. coli)	DNAK	0/2	2/4	59
21C7-8 (E. coli)	DNAK	0/2	2/4	115
17C10-31 (E. coli)	CT858	0/2	2/4	114

EXAMPLE 9

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PROTECTION STUDIES USING CHLAMYDIA ANTIGENS

Protection studies were conducted in mice to determine whether immunization with chlamydial antigens can impact on the genital tract disease resulting from chlamydial inoculation. Two models were utilized; a model of intravaginal inoculation that uses a human isolate containing a strain of *Chlamydia psittaci* (MTW447), and a model of intrauterine inoculation that involves a human isolate identified as *Chlamydia trachomatis*, serovar F (strain NI1). Both strains induce inflammation in the upper genital tract, which resemble endometritis and salpingitis caused by *Chlamydia trachomatis* in women. In the first experiment, C3H mice (4)

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mice per group) were immunized three times with 100 µg of pcDNA-3 expression vector containing C. trachomatis SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5). Inoculations were at the base of the tail for systemic immunization. Two weeks after the last immunization, animals were progesterone treated and infected, either thru the vagina or by injection of the inoculum in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored (from + for very mild, to +++++ for very severe). Scores attributed to each single oviduct /ovary were summed and divided by the number of organs examined to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control-immunized animals receiving empty vector showed consistent inflammation with an ovary /oviduct mean inflammation score of 6.12, in contrast to 2.62 for the DNA-immunized group. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 8.37, versus 5.00 for the DNA-immunized group. Also, in the later model, vaccinated mice showed no signs of tubal occlusion while negative control vaccinated groups had inflammatory cells in the lumen of the oviduct

In a second experiment, C3H mice (4 mice per group) were immunized three times with 50 µg of pcDNA-3 expression vector containing *C. trachomatis* SWIB DNA (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5) encapsulated in Poly Lactide co-Glycolide microspheres (PLG); immunizations were made intra-peritoneally. Two weeks after the last immunization, animal were progesterone treated and infected by inoculation of *C. psittaci* in the vagina. Two weeks after infection, mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. Inflammation level was scored as previously described. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean of inflammation for the group. Negative control-immunized animals receiving PLG-encapsulated empty vector showed consistent infammation with an ovary /oviduct mean inflammation score of 7.28, versus 5.71 for the PLG-encapsulated DNA immunized group. Inflammation in the peritoneum was 1.75 for the vaccinated group versus 3. 75 for the control.

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In a third experiment, C3H mice (4 per group) were immunized three times with 10 µg of purified recombinant protein, either SWIB (SEQ ID NO: 1, with the corresponding amino acid sequence provided in SEQ ID NO: 5, or S13 (SEQ ID NO: 4, with the corresponding amino acid sequence provided in SEQ ID NO: 12) mixed with Cholera Toxin (CT); the preparation was administred intranasally upon anaesthesia

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in a 20 uL volume. Two weeks after the last immunization, animal were progesterone treated and infected, either by vaginal inoculation of *C. psittaci* or by injection of *C. trachomatis* serovar F in the uterus. Two weeks after infection, the mice were sacrificed and genital tracts sectioned, stained and examined for histopathology. The degree of inflammation was scored as described above. Scores attributed to each single oviduct /ovary were summed and divided by the number of examined organs to get a mean score of inflammation for the group. In the model of uterine inoculation, negative control- immunized animals receiving cholera toxin alone showed an ovary /oviduct mean inflammation score of 4.25 (only 2 mice analyzed; 2 other died) versus 5.00 for the s13 plus cholera toxin-immunized group, and 1.00 for the SWIB plus cholera toxin. Untreated infected animals had an ovary /oviduct mean inflammation score of 7. In the model of vaginal inoculation and ascending infection, negative control-immunized mice had an ovary /oviduct mean inflammation score of 7.37 versus 6.75 for the s13 plus cholera toxin-immunized group and 5.37 for the SWIB plus cholera toxin-immunized group. Untreated infected animals had an ovary /oviduct mean inflammation score of 8.

The three experiments described above suggest that SWIB-specific protection is obtainable. This protective effect is more marked in the model of homologous infection but is still present when in a heterologous challenge infection with *C. psittaci*.

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EXAMPLE 10

PMP/RA12 FUSION PROTEINS

Various Pmp/Ra12 fusion constructs were generated by first synthesizing PCR fragments of a Pmp gene using primers containing a Not I restriction site. Each PCR fragment was then ligated into the NotI restriction site of pCRX1. The pCRX1 vector contains the 6HisRa12 portion of the fusion. The Ra12 portion of the fusion construct encodes a polypeptide corresponding to amino acid residues 192-323 of *Mycobacterium tuberculosis* MTB32A, as described in U.S. Patent Application 60/158,585, the disclosure of which is incorporated herein by reference. The correct orientation of each insert was determined by its restriction enzyme pattern and its sequence was verified. Multiple fusion constructs were made for PmpA, PmpB, PmpC, PmpF and PmpH, as described further below:

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PMPA FUSION PROTEINS

PmpA is 107 kD protein containing 982 aa and was cloned from serovar E. The PmpA protein was divided into 2 overlapping fragments, the PmpA(N-terminal) and (C-terminal) portions.

PmpA(N-term) was amplified by the sense and antisense primers:

GAGAGCGCCCCTCATGTTTATAACAAAGGAACTTATG (SEQ ID NO:306)

GAGAGCGCCGCTTACTTAGGTGAGAAGAAGGGAGTTTC (SEQ ID NO:307)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 308, encoding a 66 kD protein (619aa) expressing the segment 1-473 aa of PmpA. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 309.

PmpA(C-term) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCCATTCTATTCATTTCTTTGATCCTG (SEQ ID NO:310)

GAGAGCGGCCGCTTAGAAGCCAACATAGCCTCC (SEQ ID NO:311)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 312, encoding a 74 kD protein (691aa) expressing the segment 438-982 aa of PmpA. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 313.

PMPF FUSION PROTEINS

PmpF is 112 kD protein containing 1034 aa and was cloned from the serovar E. PmpF protein was divided into 2 overlapping fragments, the PmpF(N- term) and (C-term) portions.

PmpF(N-term) was amplified by the sense and antisense primers:

GAGAGCGCCCCTCATGATTAAAAGAACTTCTCTATCC (SEQ ID NO:314)

30 GAGAGCGGCCGCTTATAATTCTGCATCATCTTCTATGGC (SEQ ID NO:315)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 316, encoding a 69 kD protein (646aa) expressing the segment 1-499 aa of PmpF. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 317.

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PmpF(C-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGACATACGAACTCTGATGGG (SEQ ID NO:318)

GAGAGCGGCCGCTTAAAAGACCAGAGCTCCTCC (SEQ ID NO:319)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 320, encoding a 77 kD protein (715aa) expressing the segment 466-1034aa of PmpF. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 321.

PMPH FUSION PROTEINS

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PmpH is 108 kD protein containing 1016 as and was cloned from the serovar E. PmpH protein was divided into 2 overlapping fragments, the PmpH(N-term)and (C-term)portions.

PmpH(N-term) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCATGCCTTTTTCTTTGAGATCTAC (SEQ ID NO:322)

GAGAGCGGCCGCTTACACAGATCCATTACCGGACTG (SEQ ID NO:323)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 324, encoding a 64 kD protein (631aa) expressing the segment 1-484 aa of PmpH. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 325.

PmpH(C-term) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGATCCTGTAGTACAAAATAATTCAGC (SEQ ID NO:326)

25 GAGAGCGGCCGCTTAAAAGATTCTATTCAAGCC (SEQ ID NO:327)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 328, encoding a 77 kD protein (715aa) expressing the segment 449-1016aa of PmpH. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 329.

PMPB FUSION PROTEINS

PmpB is 183 kD protein containing 1750 as and was cloned from the serovar E. PmpB protein was divided into 4 overlapping fragments, PmpB(1), (2), (3) and (4).

PmpB(1) was amplified by the sense and antisense primers:

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GAGAGCGGCCGCTCATGAAATGGCTGTCAGCTACTGCG (SEQ ID NO:330)

GAGAGCGGCCGCTTACTTAATGCGAATTTCTTCAAG (SEQ ID NO:331)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 332, and encodes is a 53 kD protein (518aa) expressing the segment 1-372 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 333.

PmpB(2) was amplified by the sense and antisense primers:

GAGAGCGCCCCTCGGTGACCTCTCAATTCAATCTTC (SEQ ID NO:334)

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GAGAGCGGCCGCTTAGTTCTCTGTTACAGATAAGGAGAC (SEQ ID NO:335)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 336 and encodes a 60 kD protein (585aa) expressing the segment 330-767 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 337.

PmpB(3) was amplified by the sense and antisense primers:

GAGAGCGCCCCCCCCACCAACTGAATATCTCTGAGAAC (SEQ ID NO:338)

GAGCGGCCGCTTAAGAGACTACGTGGAGTTCTG (SEQ ID NO:339)

respectively. The resulting fusion has a DNA sequence set forth in SEQ ID NO: 340 encodes a 67 kD protein (654aa) expressing the segment 732-1236 aa of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 341

PmpB(4) was amplified by the sense and antisense primers:

25 GAGAGCGGCCGCTCGGAACTATTGTGTTCTCTTCTG (SEQ ID NO:342)

GAGAGCGCCGCTTAGAAGATCATGCGAGCACCGC (SEQ ID NO:343)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 344 encodes a 76 kD protein (700aa) expressing the segment 1160-1750 of PmpB. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 345.

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PMPC FUSION PROTEINS

PmpC is 187 kD protein containing 1774 aa and was cloned from the serovar E/L2. PmpC protein was divided into 3 overlapping fragments, PmpC(1), (2) and (3).

PmpC(1) was amplified by the sense and antisense primers:

GAGAGCGCCCCTCATGAAATTTATGTCAGCTACTGC (SEQ ID NO:346)

GAGAGCGGCCGCTTACCCTGTAATTCCAGTGATGGTC (SEQ ID NO:347)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 348 and encodes a 51 kD protein (487aa) expressing the segment 1-340 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 349.

PmpC(2) was amplified by the sense and antisense primers:

15 GAGAGCGGCCGCTCGATACACAAGTATCAGAATCACC (SEQ ID NO:350)

GAGAGCGCCGCTTAAGAGGACGATGAGACACTCTCG (SEQ ID NO:351)

respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 352 and encodes a 60 kD protein (583aa) expressing the segment 305-741 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 353.

PmpC(3) was amplified by the sense and antisense primers:

GAGAGCGGCCGCTCGATCAATCTAACGAAAACACAGACG (SEQ ID NO:354)

25 GAGAGCGGCCGCTTAGACCAAAGCTCCATCAGCAAC (SEQ ID NO:355)

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respectively. The resulting fusion construct has a DNA sequence set forth in SEQ ID NO: 356 and encodes a 70 kD protein (683aa) expressing the segment 714-1250 aa of PmpC. The amino acid sequence of the fusion protein is set forth in SEQ ID NO: 357.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

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CLAIMS

1. An isolated polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-290; (b) sequences complementary to a sequence of (a); and (c) polynucleotide sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.

- 2. The polypeptide of claim 1 wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 175-180, 189-196, 264 and 266.
- 3. An isolated polynucleotide molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1 and 2.
- 4. A recombinant expression vector comprising a polynucleotide molecule according to claim 3.
 - 5. A host cell transformed with an expression vector according to claim 4.
- 6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cells.
- 7. A fusion protein comprising a polypeptide according to any one of claims 1 and 2.
- 8. A fusion protein according to claim 7, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.
- 9. A fusion protein according to claim 7, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.
- 10. A fusion protein according to claim 7, wherein the fusion protein comprises an affinity tag.

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- 11. An isolated polynucleotide encoding a fusion protein according to claim 7.
- 12. An isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.
- 13. A pharmaceutical composition comprising a polypeptide according to claim 1, and a physiologically acceptable carrier.
- 14. A pharmaceutical composition comprising a polynucleotide molecule according to claim 3 and a physiologically acceptable carrier.
- physiologically acceptable carrier, wherein the polypeptide is encoded by polynucleotide molecule selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- and a physiologically acceptable carrier, wherein the polynucleotide molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 17. A pharmaceutical composition comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:
 - (a) a fusion protein according to claim 7;
 - (b) a polynucleotide according to claim 11; and
 - (c) an antibody according to claim 12.
- 18. A vaccine comprising a polypeptide according to claim 1, and an immunostimulant.

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- 19. A vaccine comprising a polynucleotide molecule according to claim 3 and an immunostimulant.
- 20. A vaccine comprising a polypeptide and an immunostimulant, wherein the polypeptide is encoded by a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 21. A vaccine comprising a DNA molecule and an immunostimulant, wherein the DNA molecule comprises a sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; (b) sequences complementary to a sequence of (a); and (c) sequences that hybridize to a sequence of (a) or (b) under moderately stringent conditions.
- 22. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:
 - (a) a fusion protein according to claim 7;
 - (b) a polynucleotide according to claim 11; and
 - (c) an antibody according to claim 12.
- 23. The vaccine of any one of claims 18-22 wherein the immunostimulant is an adjuvant.
- 24. A method for inducing protective immunity in a patient, comprising administering to a patient a pharmaceutical composition according to any one of claims 13-17.
- 25. A method for inducing protective immunity in a patient, comprising administering to a patient a vaccine according to any one of claims 18-22.
- 26. An isolated polyclonal antibody, or antigen-binding fragment thereof, that specifically binds to a Chlamydia protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence according to claim 1, or a complement of any of the foregoing polynucleotide sequences.

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- 27. A method for detecting Chlamydia infection in a patient, comprising:
- (a) obtaining a biological sample from the patient;
- (b) contacting the sample with a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291. (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - (c) detecting the presence of antibodies that bind to the polypeptide.
 - 28. A method for detecting Chlamydia infection in a patient, comprising:
 - (a) obtaining a biological sample from the patient;
- (b) contacting the sample with a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - (c) detecting the presence of antibodies that bind to the fusion protein.
- 29. The method of any one of claims 27 and 28 wherein the biological sample is selected from the group consisting of whole blood, serum, plasma, saliva, cerebrospinal fluid and urine.
- 30. A method for detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain reaction, wherein at least one of the oligonucleotide primers is specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; and
- (b) detecting in the sample a polynucleotide sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting *Chlamydia* infection.

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- 31. The method of claim 30, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 32. A method for detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the sample with one or more oligonucleotide probes specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291; and
- (b) detecting in the sample a polynucleotide sequence that hybridizes to the oligonucleotide probe, thereby detecting *Chlamydia* infection.
- 33. The method of claim 32 wherein the probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 34. A method for detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the biological sample with a binding agent which is capable of binding to a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting *Chlamydia* infection in the biological sample.
- 35. A method of detecting *Chlamydia* infection in a biological sample, comprising:
- (a) contacting the biological sample with a binding agent which is capable of binding to a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences

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complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and

- (b) detecting in the sample a polypeptide that binds to the binding agent, thereby detecting *Chlamydia* infection in the biological sample.
- 36. The method of any one of claims 34 and 35 wherein the binding agent is a monoclonal antibody.
- 37. The method of any one of claims 34 and 35 wherein the binding agent is a polyclonal antibody.
- 38. The method of any one of claims 34 and 35 wherein the biological sample is selected from the group consisting of whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine.
 - 39. A diagnostic kit comprising:
- (a) a polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291, (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - (b) a detection reagent.
 - 40. A diagnostic kit comprising:
- (a) a fusion protein comprising a polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions; and
 - (b) a detection reagent.
- 41. The kit of claims 39 or 40 wherein the polypeptide is immobilized on a solid support.

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- 42. The kit of claims 39 or 40 wherein the detection reagent comprises a reporter group conjugated to a binding agent.
- 43. The kit of claim 42 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.
- 44. The kit of claim 42 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.
- 45. A diagnostic kit comprising at least two oligonucleotide primers, at least one of the oligonucleotide primers being specific for a polynucleotide molecule comprising a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 46. A diagnostic kit according to claim 43, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a sequence of SEO ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 47. A diagnostic kit comprising at least one oligonucleotide probe, the oligonucleotide probe being specific for a polynucleotide molecule comprising a sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
- 48. A kit according to claim 47, wherein the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a polynucleotide sequence of SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291.
 - 49. A diagnostic kit comprising:
- (a) at least one antibody, or antigen-binding fragment thereof, according to claim 22; and
 - (b) a detection reagent.
- 50. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
 - (a) obtaining peripheral blood cells from the patient;

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- (b) incubating the cells in the presence of at least one polypeptide, the polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said antigen comprises an amino acid sequence encoded by a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
 - (c) administering to the patient the proliferated T cells.
- 51. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
 - (a) obtaining peripheral blood cells from the patient;
- (b) incubating the cells in the presence of at least one polynucleotide, comprises a polynucleotide sequence selected from the group consisting of: (i) a sequence recited in SEQ ID NO: 169-174, 181-188, 263, 265 and 267-291 (ii) sequences complementary to a sequence of (i), and (c) polynucleotide sequences that hybridize to a sequence of (i) or (ii) under moderately stringent conditions, such that T cells proliferate; and
 - (c) administering to the patient the proliferated T cells.
- 52. The method of any one of claims 50 and 51 wherein the step of incubating the T cells is repeated one or more times.
- 53. The method of any one of claims 50 and 51 wherein step (a) further comprises separating T cells from the peripheral blood cells, and the cells incubated in step (b) are the T cells.
- 54. The method of any one of claims 50 and 51 wherein step (a) further comprises separating CD4+ cells or CD8+ T cells from the peripheral blood cells, and the cells proliferated in step (b) are CD4+ or CD8+ T cells.
- 55. The method of any one of claims 50 and 51 wherein step (a) further comprises separating gamma/delta T lymphocytes from the peripheral blood cells, and the cells proliferated in step (b) are gamma/delta T lymphocytes.
- 56. The method of any one of claims 50 and 51 wherein step (b) further comprises cloning one or more T cells that proliferated in the presence of the polypeptide.

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- 57. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polypeptide of claim 1, in combination with a physiologically acceptable carrier.
- 58. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising T cells proliferated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 59. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
- (a) incubating antigen presenting cells in the presence of at least one polypeptide of claim 1;
 - (b) administering to the patient the incubated antigen presenting cells.
- 60. A method for treating *Chlamydia* infection in a patient, comprising the steps of:
- (a) introducing at least one polynucleotide of claim 3 into antigen presenting cells;
 - (b) administering to the patient the antigen presenting cells.
- 61. The method of claims 59 or 60 wherein the antigen presenting cells are selected from the group consisting of dendritic cells. macrophage cells, B cells fibroblast cells, monocyte cells, and stem cells.
- 62. A pharmaceutical composition for the treatment of *Chlamydia* infection in a patient, comprising antigen presenting cells incubated in the presence of a polypeptide of claim 1, in combination with a physiologically acceptable carrier.
- 63. A pharmaceutical composition for the treatment if *Chlamydia* infection in a patient, comprising antigen presenting cells incubated in the presence of a polynucleotide of claim 3, in combination with a physiologically acceptable carrier.
- 64. A polypeptide comprising an immunogenic portion of a *Chlamydia* antigen, wherein said immunogenic portion comprises a sequence of SEQ ID NO: 246, 247 and 254-256.

- 65. An immunogenic epitope of a *Chlamydia* antigen, comprising a sequence of SEQ ID NO: 246, 247 or 254-256.
- 66. An isolated polypeptide comprising a sequence recited in any one of SEQ ID NO: 224-262, 246, 247, 254-256, 292 and 294-305.
- 67. A recombinant fusion polypeptide comprising a an amino acid sequence of a Ra12 polypeptide and an amino acid sequence of a Chlamydial polypeptide.
- 68. The recombinant polypeptide of claims 67, wherein the Chlamydial polypeptide is a Pmp polypeptide.
- 69. The recombinant polypeptide of claims 67, wherein the Chlamydial polypeptide is a PmpA, PmpF, PmpH, PmpB, or PmpC.
- 70. The recombinant polypeptide of claims 67, wherein the amino acid sequence of the fusion polypeptide is a sequence selected from the group consisting of SEQ ID NOs: 309, 313, 317, 321, 325, 329, 333, 337, 341, 345, 349, 353 and 357.
- 71. A recombinant DNA molecule encoding a fusion polypeptide according to claim 67.

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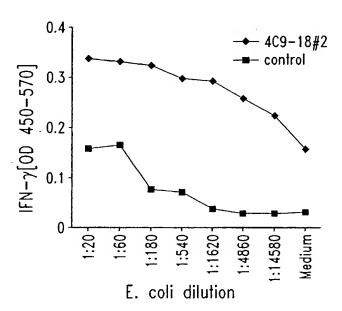
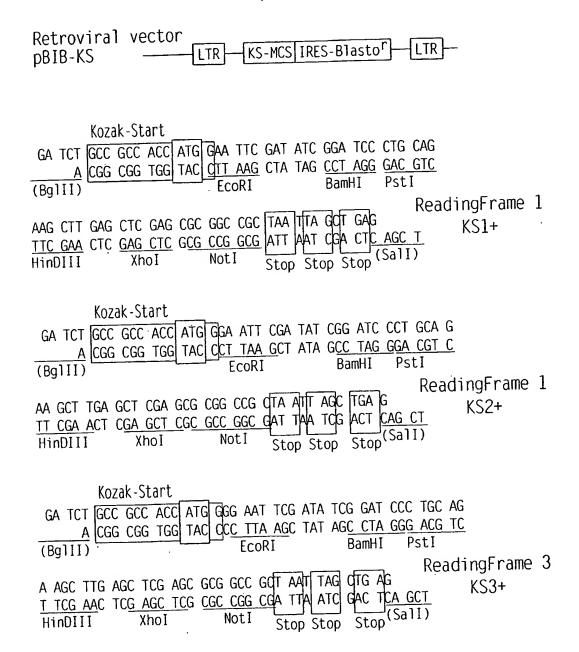


Fig. 1

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Chlamydia C17.8 Peptide Screen

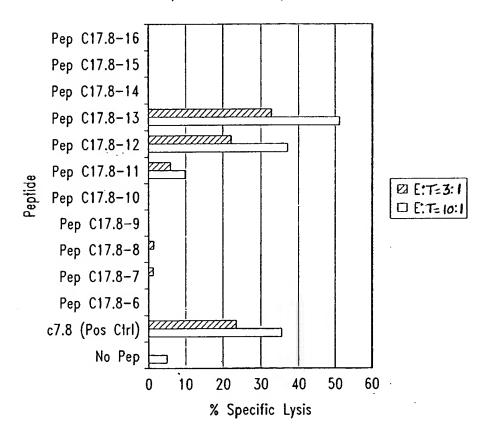
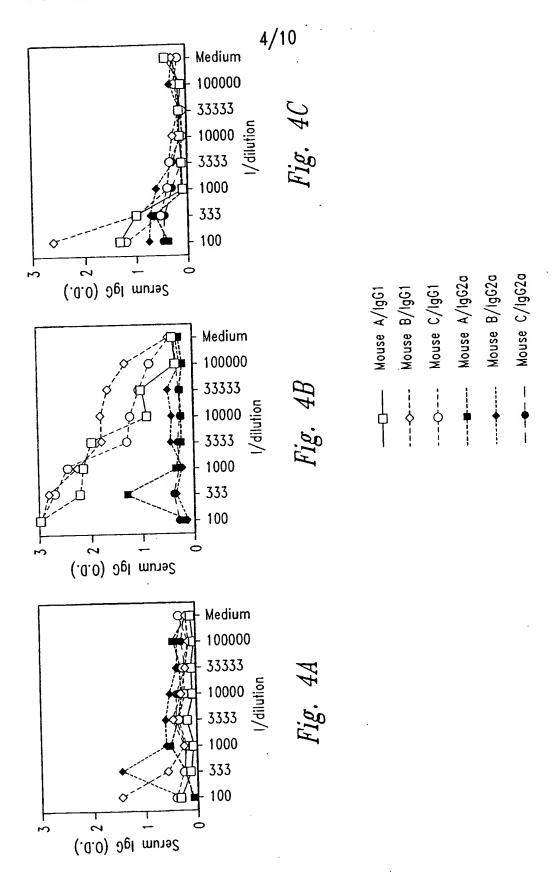
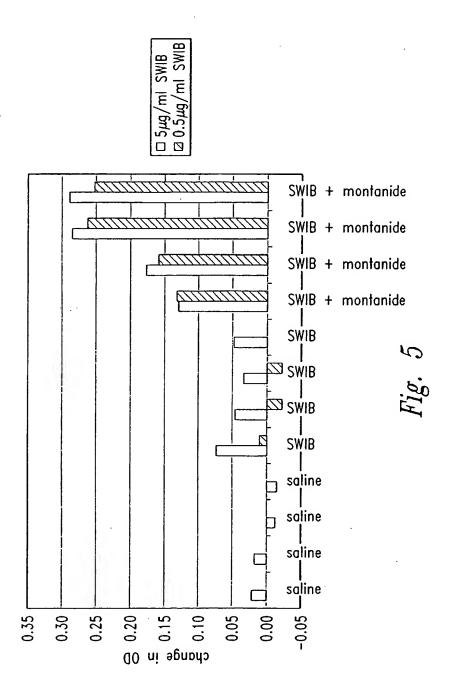


Fig. 3





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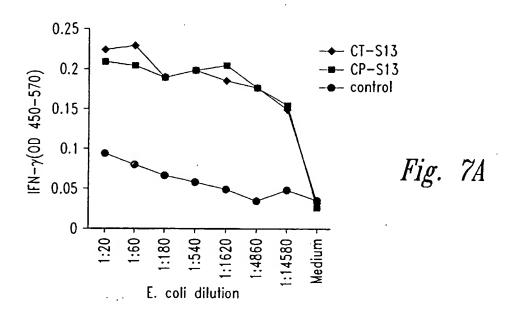
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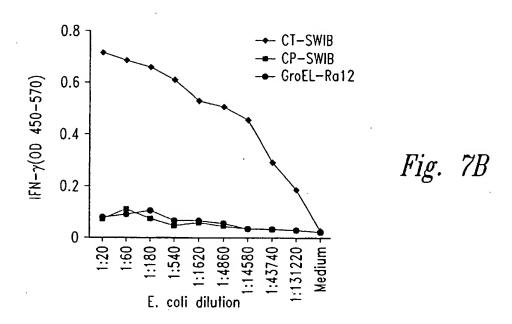
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Fig. 6

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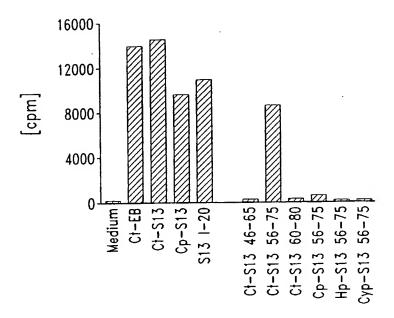
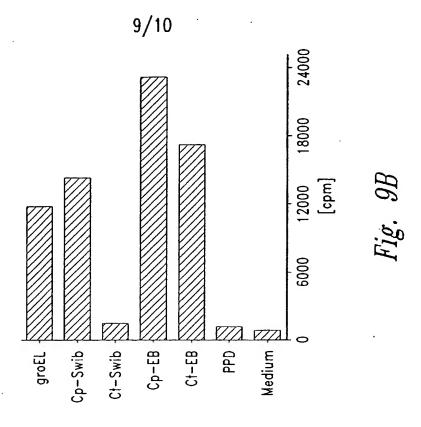
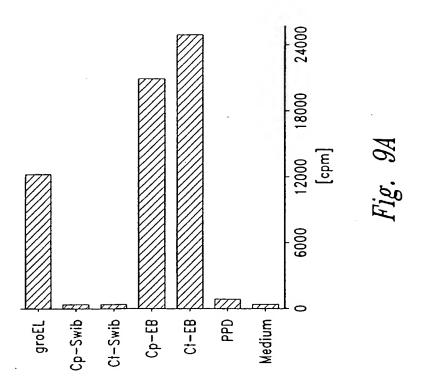
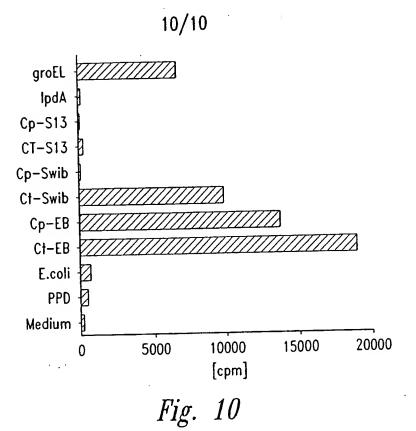


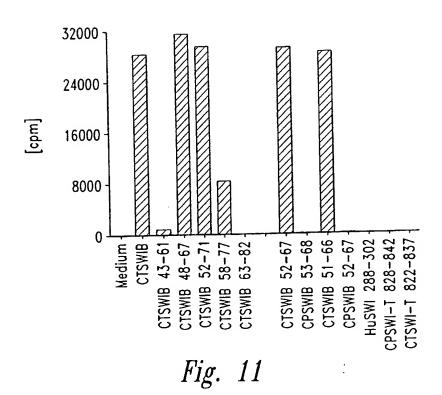
Fig. 8





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PCT/US00/32919 1

SEQUENCE LISTING

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9

165 170 Ala Val Pro Ser Val Ile Phe Thr Phe Pro Glu Val Ala Ser Val Gly 180 185 Leu Ser Pro Thr Ala Ala Gln Gln His Leu Leu Leu Arg Leu Leu Phe 200 195 205 Leu Lys Asn Leu Ile Gln Lys Lys Asn Ser Ser His Thr Cys Glu Glu 220 Glu Gly Val Trp Lys Thr Ser 225 230 <210> 27 <211> 264 <212> DNA <213> Chlamydia pneumoniae atgagtcaaa aaaataaaaa ctctgctttt atgcatcccg tgaatatttc cacagattta gcagttatag ttggcaaggg acctatgccc agaaccgaaa ttgtaaagaa agtttgggaa 120 tacattaaaa aacacaactg tcaggatcaa aaaaataaac gtaatatcct tcccgatgcg 180 aatottgcca aagtotttgg ctctagtgat cctatcgaca tgttccaaat gaccaaagcc 240 ctttccaaac atattgtaaa ataa 264 <210> 28 <211> 87 <212> PRT <213> Chlamydia pneumoniae <400> 28 Met Ser Gln Lys Asn Lys Asn Ser Ala Phe Met His Pro Val Asn Ile 1 5 10 Ser Thr Asp Leu Ala Val Ile Val Gly Lys Gly Pro Met Pro Arg Thr 20 25 Glu Ile Val Lys Lys Val Trp Glu Tyr Ile Lys Lys His Asn Cys Gln 40 45 Asp Gln Lys Asn Lys Arg Asn Ile Leu Pro Asp Ala Asn Leu Ala Lys 55 Val Phe Gly Ser Ser Asp Pro Ile Asp Met Phe Gln Met Thr Lys Ala 70 75 Leu Ser Lys His Ile Val Lys 85 <210> 29 <211> 369 <212> DNA <213> Chlamydia pneumoniae <400> 29 atgecaegea teattggaat tgatatteet geaaagaaaa agttaaaaat aagtetgaea 60 tatatttatg gaataggatc agctcgttct gatgaaatca ttaaaaagtt gaagttagat 120 cctgaggcaa gagcctctga attaactgaa gaagaagtag gacgactgaa ctctctgcta 180 caatcagaat ataccgtaga aggggatttg cgacgtcgtg ttcaatcgga tatcaaaaga 240 ttgatcgcca tccattctta tcgaggtcag agacatagac tttctttacc agtaagagga 300 caacgtacaa aaactaattc tcgtactcga aaaggtaaaa gaaaaacagt cgcaggtaag 360 369 aagaaataa <210> 30

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Ile Ile Lys Lys Leu Lys Leu Asp Pro Glu Ala Arg Ala Ser Glu Leu
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                          40
Thr Glu Glu Glu Val Gly Arg Leu Asn Ser Leu Leu Gln Ser Glu Tyr
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Thr Val Glu Gly Asp Leu Arg Arg Arg Val Gln Ser Asp Ile Lys Arg
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Leu Ile Ala Ile His Ser Tyr Arg Gly Gln Arg His Arg Leu Ser Leu
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Lys Ala Asn Met Gly
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ggaatateet gaaaagtgta eggaagaaca gattegtaca ttattggetg cagateatee 420
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gatagtacag tccaagatat tttagacaaa atcacaacag accettetet aggtttgttg 180
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ggagcaatcc aacattctct cctgcacgac cttctgggag ttcttttctg aacattcaa 180
ccccagtaac aatcgtttct ttagtatctc taagaccgac caactgaact ttatcggaaa 240
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<211> 715

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ataceggete taccatteaa gagtteeage cetateteet tettaetaat tttgggtatt 180
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ataggcatca cccacacgac taacattcgg ttttgggagt ttaataaaga gttccgtcga 600
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 cetgattete taccagteeg egtteetgea agtttegata gaaatettge acaatageag 180
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<222> (546)
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gacttattac ggaacgagta aggcggagat ttctagagtt ctgcaaaagg gtaagcactg 180
catagoogtg attgatgtac aaggagottt ggototgaag aagcaaatgo oggoagtoac 240
tatttttatt caageteet etcaagaaga acttgagege egtttgaatg etegggatte 300
aqaqaaaqat ttccaqaaqa aaqaaagatt agaqcatagc gctgtcgaaa ttgctgccgc 360
aagtattttt atagctgaag aacataggat gagtcatggn tagaaaagat cgtttaacta 480
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<210> 74
<211> 465
<212> DNA
<213> Chlamydia
<400> 74
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caatgeggeg tggagtaetg ggtateggge tgtgtttggta tggattttet ccattacaca 180
actatatagg atcgctagat tgtttcggtc gtcccttaca gatgacgcaa agtaatcttg 240
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<211> 545
<212> DNA
<213> Chlamydia
<400> 75
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ctatatgttc tgtgaaatat gcatagtctt caggattgga aaatccaaag tactcagtca 180
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545
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<211> 285
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<213> Chlamydia
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attgtaaaga aagtttggga atacattaaa aaacacaact gtcaggatca aaaaaataaa 180
cgtaatatcc ttcccgatgc gaatcttgcc aaagtctttg gctctagtga tcctatcgac 240
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<210> 79

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<211> 950
<212> DNA
<213> Chlamydia
<400> 79
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<211> 395
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<213> Chlamydia
<400> 80
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Ile Lys Lys His Asn Cys Gln Asp Gln Lys Asn Lys Arg Asn Ile Leu
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Val Ile Phe Thr Phe Pro Glu Val Ala Ser Val Gly Leu Ser Pro Thr 355 360 365

Ala Ala Gln Gln Lys Ile Pro Val Lys Val Thr Lys Phe Pro Phe 370 375 380

Arg Ala Ile Gly Lys Ala Val Ala Met Gly Glu Ala Asp Gly Phe Ala 385 390 395 400

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Thr Pro Leu His Met Pro Pro Ala Lys Lys 465 470

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Glu Ala Arg Ala Ser Glu Leu Thr Glu Glu Glu Val Gly Arg Leu Asn 50 55 60

Ser Leu Leu Gln Ser Glu Tyr Thr Val Glu Gly Asp Leu Arg Arg 65 70 75 80

Val Gln Ser Asp Ile Lys Arg Leu Ile Ala Ile His Ser Tyr Arg Gly 85 90 95

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Lys

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Lys Asp Phe Thr Tyr Val Cys Pro Thr Glu Leu His Ala Phe Gln Asp 55

Arg Leu Val Asp Phe Glu Glu His Gly Ala Val Val Leu Gly Cys Ser

Val Asp Asp Ile Glu Thr His Ser Arg Trp Leu Thr Val Ala Arg Asp

Ala Gly Gly Ile Glu Gly Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser 105

Phe Lys Ile Ser Glu Ala Phe Gly Val Leu Asn Pro Glu Gly Ser Leu 120

Ala Leu Arg Ala Thr Phe Leu Ile Asp Lys His Gly Val Ile Arg His

Ala Val Ile Asn Asp Leu Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu 155 150

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Δla	-	Tle	Thr	Δla	Ser		Val	Ser	Lvs	Glv		Glv	Δsn	Ala	Δrα	
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His	_	Arg	Arg	Ala	Ala		Ala	Val	Cys	Ser		Ile	GIY	Gly	Ile	
The	130	Lou	. ה	Th∽	Dho	135	777	Tla	7~~	Dro	140	T 011	Dho	1751	7 on	
145	ıyı	пец	Ala	1111	150	GIY	Ala	116	Arg	155	116	Deu	FIIC	Val	160	
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Asn	Ala	Pne		Thr	Arg	PIO	GIY	25	БуБ	1100	501	5	30		
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WO 01/40474 51 PCT/US00/32919

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	БУЗ	ASII	TYL	GLY	310	AIG	110	LYL	ΑIG	315	Vai	Val	1111	DCu	320
305	2	a1	D	mb ~		Dha	т1 -	7.00	7 ~~		212	7.00	7 ~ ~	Tura	
Asp	ASI	GIY	Pro		Tyt	Pne	116	Asn		116	Ala	ASII	ASII		Gry
		_		325				_	330		_		_	335	_
_			340			_		Ser 345					350		
Arg	His	Ala	Ile	Ile	Phe	Asn	Glu	Asn	Ile	Val	Thr	Asn	Val	Thr	Asn
		355					360					365			
Ala	Asn 370	Gly	Thr	Ser	Thr	Ser 375	Ala	Asn	Pro	Pro	Arg 380	Arg	Asn	Ala	Ile
Thr		Ala	Ser	Ser	Ser	Glv	Glu	Ile	Leu	Leu	Glv	Ala	Glv	Ser	Ser
385					390	2				395	2		- 4		400
	λen	T.OU	Tle	Dhe		λen	Pro	Ile	Glu		Ser	Δen	Δla	Glv	
GIII	MSII	DCu	110	405	I y I	nsp	110	110	410	• 441	001		7114	415	• • • •
C ~ ~	7707	C 0 x	Dho		7	C1,,	ח ז ח	Asp		Thr	Clv	Car	นาไ		Dho
Ser	vai	ser		ASII	ьys	Gru	ATG	_	GIII	1111	Gry	Ser		vai	FILE
_			420		_	_		425	_,		~ 3		430		~ 3
Ser	GTA		Thr	Val	Asn	ser		Asp	Pne	His	GIN		Asn	ьeu	Gin
Thr	Lys	435 Thr	Pro	Ala	Pro	Leu	440 Thr	Leu	Ser	Asn	Gly	445 Phe	Leu	Cys	Ile
	450					455					460				
Glu 465	Asp	His	Ala	Gln	Leu 470	Thr	Val	Asn	Arg	Phe 475	Thr	Gln	Thr	Gly	Gly 480
Val	Val	Ser	Leu	Glv	Asn	Gly	Ala	Val	Leu	Ser	Cys	Tyr	Lys	Asn	Gly
				485		- 2			490		•	•	-	495	•
Thr	Gly	Asp	Ser 500		Ser	Asn	Ala	Ser 505		Thr	Leu	Lys	His 510	_	Gly
T.011	7 en	T.011		Ser	Tle	Len	Lvs	Ser	Glv	Δla	Glu	Tle		Leu	Leu
Бец	ASII	515	361	SEL	116	ьси	520	JCI	GIY	AIU	OIU	525	110	204	шеш
m	3703		Dro	Th.	N am	Non		Asn	A cn	T1 17	Th~		7.00	Thr	λla
пр		GIU	PIO	1111	ASII		SEL	Maii	ASII	IYL		мта	ASP	1111	AIG
	530		_	_	_	535		_	_	_	540		_	_	
	Thr	Phe	Ser	Leu		Asp	Val	Lys	Leu		Leu	He	Asp	Asp	
545					550					555					560
Gly	Asn	Ser	Pro	Tyr	Glu	Ser	Thr	Asp	Leu	Thr	His	Ala	Leu	Ser	Ser
				565					570					575	
Gln	Pro	Met	Leu	ser	Ile	Ser	Glu	Ala	Ser	Asp	Asn	Gln	Leu	Gln	Ser
			580					585		_			590		
~-3									_					m	C1-
GIU	Asn	Ile	Asp	Phe	Ser	Gly	ьeu	Asn	Val	Pro	His	Tyr	GIV	Trp	GIII
GIU	Asn		Asp	Phe	Ser	GIY		Asn	Val	Pro	His		GIY	Trp	GIII
		595					600	Asn Lys				605			

	610					615					620				
Ser	Ser	Ala	Thr	Ile	Thr	Asp	Pro	Gln	Lys	Ala	Asn	Arg	Phe	His	Arg
625					630	_			_	635		_			640
Thr	Leu	Leu	Leu	Thr	Trp	Leu	Pro	Ala	Gly	Tyr	Val	Pro	Ser	Pro	Lys
				645	_				650	•				655	•
His	Arq	Ser	Pro	Leu	Ile	Ala	Asn	Thr	Leu	Trp	Gly	Asn	Met	Leu	Leu
			660					665		•	•		670		
Ala	Thr	Glu	Ser	Leu	Lvs	Asn	Ser		Glu	Leu	Thr	Pro	Ser	Glv	His
-		675	-		•		680					685		3	
Pro	Phe	Tro	Glv	Ile	Thr	Glv		Glv	Leu	Glv	Met	Met	Val	Tvr	Gln
	690	-1-	- 2			695	2	2		2	700		–	- 2 -	
Asp		Ara	Glu	Asn	His		Glv	Phe	His	Met		Ser	Ser	Glv	Tvr
705		5			710		2			715				1	720
–	Ala	Glv	Met	Ile		Glv	Gln	Thr	His		Phe	Ser	Leu	Lvs	
		 1		725		0-1	· · · ·		730			-		735	
Ser	Gln	Thr	Tvr		Lvs	Leu	Asn	Glu		Tvr	Δla	Lys	Asn		Val
	01		740		_,_			745	9	-1-		_,_	750		• • • •
Ser	Ser	Lvs		Tvr	Ser	Cvs	Gln		Glu	Met	Len	Phe		Leu	Gln
	501	755		-] -	001	0,0	760	OL J	O_Lu		Dou	765		Dou	0111
Glu	Glv		Leu	Leu	Thr	Lvs		Val	Glv	T.eu	Tvr	Ser	Tvr	Glv	Asn
GIG	770	2 340	Deu	Deu		775	БСС	Vu.1	017	ДСЦ	780	DCI	- 7 -	O. y	лор
His		Cvs	His	His	Dhe		Thr	Gln	Glv	Glu		Leu	Thr	Ser	Gln
785	71011	Cys	*****	1110	790	-7-		0111	O _x y	795	71011	ДСИ	****	001	800
	Thr	Dhe	Δτα	Ser		Thr	Met	Glv	Glv		Val	Phe	Dhe	Δen	
O. J	1111	1	71.9	805	0111	1111	1100	OI,	810	2114	vui	1	1110	815	Deu
Pro	Met	Lvs	Pro		Glv	Ser	Thr	His		T.eu	Thr	Ala	Pro		Leu
		2,0	820		017	001		825					830		
Glv	Ala	Leu		Tle	Tvr	Ser	Ser		Ser	His	Phe	Thr		Val	Glv
017		835	U -1		- / -		840					845		• • • •	017
Ala	Tvr		Ara	Ser	Phe	Ser		Lvs	Thr	Pro	Leu	Ile	Asn	Val	Leu
	850			001		855		ц		110	860	110	71011	·u_	Deu
Val		Tle	Glv	Val	Lvs		Ser	Phe	Met	Asn		Thr	His	Ara	Pro
865			0-1		870		001			875					880
	Δla	Tro	Thr	Val		Leu	Δla	Tvr	Gln		Val	Leu	Tvr	Ara	
				885				-1-	890				-1-	895	
Glu	Pro	Glv	Ile		Thr	Gln	Leu	Leu		Ser	Lvs	Gly	Ile		Phe
		1	900					905		001	27,5	01	910		
Glv	Ser	Glv		Pro	Ser	Ser	Ara		Δla	Met	Ser	Tyr		Tle	Ser
- -,		915					920		7114			925			
Gln	Gln		Gln	Pro	Len	Ser		Len	Thr	Len	His	Phe	Gln	Tur	His
J	930		J			935		~~u	****	~cu	940		J.1.1	- 1 -	
Glv		Tvr	Ser	Ser	Ser		Phe	Cvs	Asn	Tvr		Asn	Glv	Glu	Tle
945		- / -			950			~ <i>J</i> 5	.1011	955			J- J	Jiu	960
_	Leu	Arg	Phe												,,,,
		3													

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<212> PRT

<213> Chlamydia

<400> 178

 Met
 Ser
 Ser
 Glu
 Lys
 Asp
 Ile
 Lys
 Ser
 Thr
 Cys
 Ser
 Lys
 Phe
 Ser
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Gly	Pro 50	Gln	Ala	Val	Leu	Leu 55	Leu	Asp	Gln	Ile	Arg 60	Asp	Leu	Phe	Val
Gly 65		Lys	Asp	Ser	Gln 70	Ala	Glu	Gly	Gln	Tyr 75	Arg	Leu	Ile	Val	Gly 80
	Pro	Ser	Ser	Phe 85	Gln	Glu	Lys	Asp	Ala 90	Asp	Thr	Leu	Pro	Gly 95	Lys
Val	Glu	Gln	Ser 100		Leu	Phe	Ser	Val 105	Thr	Asn	Pro	Val	Val 110	Phe	Gln
Gly	Val	Asp 115	Gln	Gln	Asp	Gln	Val 120		Ser	Gln	Gly	Leu 125	Ile	Cys	Ser
Phe	Thr 130		Ser	Asn	Leu	Asp 135	Ser	Pro	Arg	Asp	Gly 140	Glu	Ser	Phe	Leu
Gly 145	Ile	Ala	Phe	Val	Gly 150	Asp	Ser	Ser	Lys	Ala 155	Gly	Ile	Thr	Leu	Thr 160
Asp	Val	Lys	Ala	Ser 165	Leu	Ser	Gly	Ala	Ala 170	Leu	Tyr	Ser	Thr	Glu 175	Asp
			Glu 180					185					190		
		195	Gln	_			200					205			
	210		Gly			215					220				
225			Ser		230					235					240
			Gly	245					250					255	
-	_		Val 260					265					270		
		275	Asn				280					285			
	290		Val			295					300				
Ala 305	Leu	ser	Gly	GIY	310	тте	Ala	Ala	ser	315	Asp	TTE	Ala	Pne	320
	_		Glu	325					330					335	
			Ser 340					345					350		
Leu	Leu	Gln 355	Gly	Asn	His	Gly	Ile 360	Thr	Cys	Asp	Lys	Asn 365	Glu	Ser	Ala
Ser	Gln 370	Gly	Gly	Ala	Ile	Phe 375	Gly	Lys	Asn	Суѕ	Gln 380	Ile	Ser	Asp	Asn
Glu 385		Pro	Val	Val	Phe 390	Arg	Asp	Ser	Thr	Ala 395	Cys	Leu	Gly	Gly	Gly 400
		Ala	Ala	Gln 405		Ile	Val	Ser	Ile 410		Asn	Asn	Gln	Ala 415	Gly
Ile	Ser	Phe	Glu 420		Gly	Lys	Ala	Ser 425		Gly	Gly	Gly	Ile 430	Ala	Cys
Gly	Ser	Phe 435	Ser	Ser	Ala	Gly	Gly 440		Ser	Val	Leu	Gly 445	Thr	Ile	Asp
Ile	Ser 450	Lys	Asn	Leu	Gly	Ala 455	Ile	Ser	Phe	Ser	Arg 460	Thr	Leu	Cys	Thr
	Ser	Asp	Leu	Gly		Met	Glu	Tyr	Gln	Gly 475	Gly	Gly	Ala	Leu	Phe 480
465 Gly	Glu	Asn	Ile	Ser 485	470 Leu	Ser	Glu	Asn	Ala 490		Val	Leu	Thr	Phe	
Asp	Asn	Ile	Val 500		Thr	Phe	Ala	Ser 505		Gly	Lys	Ile	Leu 510		Gly

Gly Ala Ile Leu Ala Thr Gly Lys Val Glu Ile Thr Asn Asn Ser Gly 520 Gly Ile Ser Phe Thr Gly Asn Ala Arg Ala Pro Gln Ala Leu Pro Thr 535 540 Gln Glu Glu Phe Pro Leu Phe Ser Lys Lys Glu Gly Arg Pro Leu Ser 550 555 Ser Gly Tyr Ser Gly Gly Gly Ala Ile Leu Gly Arg Glu Val Ala Ile 565 570 Leu His Asn Ala Ala Val Val Phe Glu Gln Asn Arg Leu Gln Cys Ser 585 Glu Glu Glu Ala Thr Leu Leu Gly Cys Cys Gly Gly Gly Ala Val His 600 Gly Met Asp Ser Thr Ser Ile Val Gly Asn Ser Ser Val Arg Phe Gly 615 Asn Asn Tyr Ala Met Gly Gln Gly Val Ser Gly Gly Ala Leu Leu Ser 630 635 Lys Thr Val Gln Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn 645 650 Ile Ala Ser Leu Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys 665 Glu Leu Val Asp Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg 680 Val Tyr Gly Gly Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser 695 700 Gly Asn Lys Gly Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu 710 715 Tyr Val Glu Glu Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro 730 Glu Gln Lys Asp Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln 745 Ser Phe Ile Thr Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly 760 Asp Leu Ser Pro Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg 775 780 Arg Glu Cys Ala Gly Gly Ala Ile Phe Ala Lys Arg Val Arg Ile Val 790 795 Asp Asn Gln Glu Ala Val Val Phe Ser Asn Asn Phe Ser Asp Ile Tyr 810 Gly Gly Ala Ile Phe Thr Gly Ser Leu Arg Glu Glu Asp Lys Leu Asp 825 Gly Gln Ile Pro Glu Val Leu Ile Ser Gly Asn Ala Gly Asp Val Val 840 Phe Ser Gly Asn Ser Ser Lys Arg Asp Glu His Leu Pro His Thr Gly 855 860 Gly Gly Ala Ile Cys Thr Gln Asn Leu Thr Ile Ser Gln Asn Thr Gly 870 875 Asn Val Leu Phe Tyr Asn Asn Val Ala Cys Ser Gly Gly Ala Val Arg 890 Ile Glu Asp His Gly Asn Val Leu Leu Glu Ala Phe Gly Gly Asp Ile 905 Val Phe Lys Gly Asn Ser Ser Phe Arg Ala Gln Gly Ser Asp Ala Ile 920 Tyr Phe Ala Gly Lys Glu Ser His Ile Thr Ala Leu Asn Ala Thr Glu 935 940 Gly His Ala Ile Val Phe His Asp Ala Leu Val Phe Glu Asn Leu Lys 950 955 Glu Arg Lys Ser Ala Glu Val Leu Leu Ile Asn Ser Arg Glu Asn Pro 970

Gly	Tyr	Thr	Gly 980	Ser	Ile	Arg	Phe	Leu 985	Glu	Ala	Glu	Ser	Lys 990	Val	Pro
Gln	Cys	Ile 995	His	Val	Gln	Gln	Gly 1000		Leu	Glu	Leu	Leu 1005		Gly	Ala
Thr	Leu 1010		Ser	Tyr	Gly	Phe 1015		Gln	Asp	Ala	Gly 1020		Lys	Leu	Val
	Ala		Gly	Ser	Lys 1030	Leu		Ile	Leu	Asp 1039	Ser		Thr	Pro	Val 1040
1025				_					_					_	
	Gly			1045	5			•	1050)				1055	5
	Pro		1060)				106	5				1070)	
	Val	1075	5				1080)				1089	5		
Phe	Ser 1090		Ser	Gln	Gln	Glu 1099		Thr	Val	Glu	Ala 1100		Gln	Val	Ile
Val.	Pro	Glv	Gly	Sar	ጥህን	Val	Δνα	Ser	Glv	Glu	T.e.11	Δsn	T.e.11	Glu	T.eu
		Gry	Gry	JCI	_		n. 9	JCI	CI			ASII	ьси	OIU	
1109					1110					1119					1120
	Asn			1125	5		_		1130)				1135	5
Glu	Ala	Lys	Val 1140		Leu	Met	Ser	Phe 1149		Ala	Ser	Ser	Asp 1150		Ala
Ser	Ala	Glu 1159		Ser	Asn	Leu	Ser 1160		Ser	Asp	Leu	Gln 1169		His	Val
Ala	Thr 1170		Glu	Ile	Glu	Glu 1179		Thr	Tyr	Gly	His 1180		Gly	Asp	Trp
Ser	Glu	Δla	Lvs	Tle	Gln	Asp	Glv	Thr	Leu	Val	Ile	Asn	Trp	Asn	Pro
118			-,-		1190		017			1199					1200
		_	-	-			~1.	•				T	**- 3	Db.	
Thr	Gly	Tyr	Arg		_	Pro	GIN	гуѕ			Ата	ьeu	vai		
				1209					1210					1215	
Ala	Leu	Trp	Glu 1220		Gly	Ala	Val	Leu 122		Ala	Leu	Lys	Asn 1230		Arg
Phe	Ala	His 1239		Leu	Thr	Ala	Gln 1240		Met	Glu	Phe	Asp 1245		Ser	Thr
Asn	Val 1250	_	Gly	Phe	Ala	Phe 1255		Gly	Phe	Arg	Thr 1260		Ser	Ala	Glu
Asn	Leu	Val	Ala	Ile	Asp	Gly	Tyr	Lys	Gly	Ala	Tyr	Gly	Gly	Ala	Ser
126					1270		-	-	_	1279		-	-		1280
	Gly	Val	Asp	Ile 1289	Gln		Met	Glu	Asp 1290	Phe		Leu	Gly	Val 1299	Ser
C111	Ala	ח ה	Dho		-	Tarc	Mot	7 cn			Lare	Dha	λen		
			1300)				130	5				1310)	
	Ser	1319	5				1320)				1325	5	_	
Gly	Ser 1330	-	Phe	Phe	Lys	Gly 133		Tyr	Ser	Leu	Gly 1340		Thr	GIn	Asn
Asp	Met	Lys	Thr	Arg	Tyr	Gly	Val	Leu	Gly	Glu	Ser	Ser	Ala	Ser	Trp
134	5				1350)				1355	5				1360
Thr	Ser	Ara	Glv	Val	Leu	Ala	Asp	Ala	Leu	Val	Glu	Tvr	Arg	Ser	Leu
	5 01	y	O ₁	1369			p		1370		014	-3-	5	1375	
Val	Gly	Pro	Val 1380	-	Pro	Thr	Phe	Tyr 1389		Leu	His	Phe	Asn 1390		Tyr
บลา	Glu	Val			Ala	Ser	Met			Pro	Glv	Phe			Gln
VAI	oru	139		- 7 -	AL CI	501	1400		1 116	110	O T Y	1405		014	
Gly	Arg	Glu		Arg	Ser	Phe 141	Glu		Ala	Ser	Leu 1420	Thr		Ile	Thr
71.	Pro		Gl v	Met	LMC			T.e.v	Δla	Dho			Glv	Gln	Phe
		neu	GIA	1-1CC			o _z u	Leu	viq			Lys	OTA	O 111	1440
142	5				143	J				1435	,				1440

Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr Ala Trp Glu Ala Tyr Arg 1445 1450 Lys Val Glu Gly Gly Ala Val Gln Leu Leu Glu Ala Gly Phe Asp Trp 1460 1465 1470 Glu Gly Ala Pro Met Asp Leu Pro Arg Gln Glu Leu Arg Val Ala Leu 1475 1480 1485 Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe Ser Thr Val Leu Gly Leu 1490 1495 1500 Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr Asp Ser Lys Leu Gly Tyr 1505 1510 1515 Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 1525

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Lys Asp Thr Gln Val Ser Glu Ser Pro Glu Ser Thr Pro Ser Pro Asp

300

290 295

305					310					315					320
Asp	Val	Leu	Gly	Lys 325	Gly	Gly	Gly	Ile	Tyr 330	Thr	Glu	Lys	Ser	Leu 335	Thr
Ile	Thr	Gly	Ile 340	Thr	Gly	Thr	Ile	Asp 345	Phe	Val	Ser	Asn	Ile 350	Ala	Thr
		355	Ala				360	_				365	_		
Thr	Asn 370	Ser	Leu	Gln	Phe	Leu 375	Lys	Asn	Ser	Ala	Gly 380	Gln	His	Gly	Gly
385		_	Val		390					395					400
			Thr	405				_	410					415	
		-	Gly 420		_			425					430		
		435	Lys				440					445			
	450		Ile			455					460				
465			Ser Ser		470					475					480
			Pro	485					490					495	
			500 Ser					505					510		
		515	Val	_			520					525			
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545		_	Asn	_	550					555					560
			Glu	565					570					575	
Ser	Ala	Ala	580 Lys	Glu	Gly	Gly	Val	585 Ile	His	Ser	Lys	Thr	590 Val	Thr	Leu
Ser		595 Leu	Lys	Ser	Thr		600 Thr	Phe	Ala	Asp		605 Thr	Val	Lys	Ala
	610 Val	Glu	Ser	Thr		615 Glu	Ala	Pro	Glu		620 Ile	Pro	Pro	Val	
625 Gly	Glu	Glu	Ser		630 Ala	Thr	Glu	Asn	Pro 650	635 Asn	Ser	Asn	Thr	Glu 655	640 Gly
Ser	Ser	Ala	Asn 660	645 Thr	Asn	Leu	Glu	Gly 665		Gln	Gly	Asp	Thr 670		Asp
Thr	Gly	Thr 675	Gly	Val	Val	Asn	Asn 680		Ser	Gln	Asp	Thr 685		Asp	Thr
Gly	Asn 690	Ala	Glu	Ser	Gly	Glu 695	Gln	Leu	Gln	Asp	Ser 700	Thr	Gln	Ser	Asn
Glu 705	Glu	Asn	Thr	Leu	Pro 710	Asn	Ser	Ser	Ile	Asp 715	Gļn	Ser	Asn	Glu	Asn 720
Thr	Asp	Glu	Ser	Ser 725	Asp	Ser	His	Thr	Glu 730	Glu	Ile	Thr	Asp	Glu 735	Ser
			Ser 740		-		-	745					750	_	
		755	Ser				760	_				765			
Ala	Cys	Leu	Ala	Lys	Ser	Tyr	Ala	Ala	Ser	Thr	Asp	Ser	Ser	Pro	Val

770		775		780			
Ser Asn Ser S	Ser Gly Ser 790	Asp Val 1		Ser Ser 795	Asp Asn	Pro	Asp 800
Ser Ser Ser S	Ser Gly Asp 805	Ser Ala G	Gly Asp 810	Ser Glu	Gly Pro	Thr 815	Glu
Pro Glu Ala (Gly Ser Thr 820		Thr Pro	Thr Leu	Ile Gly 830	Gly	Gly
Ala Ile Tyr (Gly Glu Thr	Val Lys I 840	Ile Glu	Asn Phe	Ser Gly 845	Gln	Gly
Ile Phe Ser 0 850	Gly Asn Lys	Ala Ile A	Asp Asn	Thr Thr 860	Glu Gly	Ser	Ser
Ser Lys Ser A	Asn Val Leu 870	Gly Gly A		Tyr Ala 875	Lys Thr	Leu	Phe 880
Asn Leu Asp S	Ser Gly Ser 885	Ser Arg A	Arg Thr 890	Val Thr	Phe Ser	Gly 895	Asn
	900	9	905		910		
Tyr Ser Pro 1 915		920			925		
Ser Ala Thr A		935		940			
Thr Phe Gly 0	950	_		955			960
Gly Ala His I	965		970			975	
	980	9	985		990		Ī
Ser Tyr Tyr I	_	1000			1005		_
Ala Pro Val V 1010		1015		102	0		
Arg Ser Leu G	103)		1035			1040
Ile Glu Ser I	1045		1050			1055	5
_	1060	1	1065		107	0	
Val Thr Lys 1 1075	ryr Gly Ala	1080	ene Giy	Gin lie	1085	ser	Asn
Gly Ser Gln 1	Thr Asp Asn	Leu Pro L 1095	Leu Lys	Leu Ile 1100		Gly	Gly
Asn Ile Cys I 1105	Phe Arg Asn 1110			Pro Thr 1115	Ser Ser	Asp	Thr 1120
Gly Thr Ser T				Asp Val	Lys Leu	Thr 1135	Met
Gln Ala Ala I	Lys Gly Lys 1140		Ser Phe	Phe Asp	Ala Ile 115		Thr
Ser Thr Lys I 1155	Lys Thr Gly	Thr Gln A	Ala Thr	Ala Tyr	Asp Thr 1165	Leu	Asp
Ile Asn Lys S	Ser Glu Asp	Ser Glu T 1175	hr Val .	Asn Ser 1180		Thr	Gly
Thr Ile Leu F 1185	Phe Ser Ser 1190			Asn Lys 1195	Ser Tyr	Ile	Pro 1200
Gln Asn Val V	1205		1210			1215	;
Glu Leu His V			_	Glu Gly	Ser Ser 123		Val
Met Thr Pro G	1220		1225				

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Ala Ala His Thr Ser Arg Thr Arg Asn Phe Ala Ala Ala Thr 1330 1335 1340	Ala
Thr Pro Thr Thr Pro Thr Ala Thr Thr Thr Ser Asn Gln 1345 1350 1355	Val 1360
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Leu Asn Asp Lys Met Asn Leu Ala Arg Phe Asp Glu Val Ser Tyr . 1475 1480 1485	
Asn Leu Trp Ile Ser Gly Leu Gly Thr Met Leu Ser Gln Val Gly 1490 1495 1500	
	1520
Leu Asp Ala Lys Pro Ala His Asp Val Ile Val Gly Ala Ala Phe : 1525 1530 1535	
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His Lys Gly Ser Glu Tyr Ser Tyr Gln Ala Ser Val Tyr Gly Gly 1555 1560 1565 Pro Phe His Phe Val Ile Asn Lys Lys Thr Glu Lys Ser Leu Pro	-
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1605 1610 1615 Leu Gly Trp Leu Thr Ala Leu Arg Val Ser Ser Val Leu Arg Thr	
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• • •	- 7 -
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Pro	Val	Thr 435		Lys	Gly	Gly	Gly 440	Leu	Tyr	Thr	Asp	Lys 445	Asn	Leu	Ser
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	_			725			Met		730					735	
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	770					775					780				
Asn 785		ггуя	sser	е сту	790		Phe	ser	ASN	795		MIG	non	261	800

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n ·	850	7	D	C	~1	855	C	T	7	17- ³	860	C1	03	- רג	T 1.
865					Gln 870					875					880
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83

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WO 01/40474

96

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710
                             715
Glu Asn His Pro Gly Phe His Met Arg Ser Ser Gly Tyr Ser Ala Gly
                730
      725
Met Ile Ala Gly Gln Thr His Thr Phe Ser Leu Lys Phe Ser Gln Thr
                       745
        740
Tyr Thr Lys Leu Asn Glu Arg Tyr Ala Lys Asn Asn Val Ser Ser Lys
                     760
Asn Tyr Ser Cys Gln Gly Glu Met Leu Phe Ser Leu Gln Glu Gly Phe
                  775
Leu Leu Thr Lys Leu Val Gly Leu Tyr Ser Tyr Gly Asp His Asn Cys
                  795 800
785 790
His His Phe Tyr Thr Gln Gly Glu Asn Leu Thr Ser Gln Gly Thr Phe
                    810
           805
Arg Ser Gln Thr Met Gly Gly Ala Val Phe Phe Asp Leu Pro Met Lys
                       825
        820
Pro Phe Gly Ser Thr His Ile Leu Thr Ala Pro Phe Leu Gly Ala Leu
     835 840
Gly Ile Tyr Ser Ser Leu Ser His Phe Thr Glu Val Gly Ala Tyr Pro
  850 855 860
Arg Ser Phe Ser Thr Lys Thr Pro Leu Ile Asn Val Leu Val Pro Ile
865 870 875 880
Gly Val Lys Gly Ser Phe Met Asn Ala Thr His Arg Pro Gln Ala Trp
        885 890
Thr Val Glu Leu Ala Tyr Gln Pro Val Leu Tyr Arg Gln Glu Pro Gly
      900 905
Ile Ala Thr Gln Leu Leu Ala Ser Lys Gly Ile Trp Phe Gly Ser Gly
915 920
Ser Pro Ser Ser Arg His Ala Met Ser Tyr Lys Ile Ser Gln Gln Thr
                  935
Gln Pro Leu Ser Trp Leu Thr Leu His Phe Gln Tyr His Gly Phe Tyr
945 950 955
Ser Ser Ser Thr Phe Cys Asn Tyr Leu Asn Gly Glu Ile Ala Leu Arg
Phe
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<210> 192

<211> 848

<212> PRT

<213> Chlamydia

<400> 192

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	130					135					140		Ser		
Glu 145	Glu	Asp	Lys	Leu	Asp 150		Gln	Ile	Pro	Glu 155	Val	Leu	Ile	Ser	Gly 160
Asn	Ala	Gly	Asp	Val 165	Val	Phe	Ser	Gly	Asn 170	Ser	Ser	Lys	Arg	Asp 175	Glu
His	Leu	Pro	His 180		Gly	Gly	Gly	Ala 185	Ile	Cys	Thr	Gln	Asn 190	Leu	Thr
Ile	Ser	Gln 195	Asn	Thr	Gly	Asn	Val 200		Phe	Tyr	Asn	Asn 205	Val	Ala	Cys
Ser	Gly 210	Gly	Ala	Val	Arg	Ile 215		Asp	His	Gly	Asn 220	Val	Leu	Leu	Glu
Ala 225	Phe	Gly	Gly	Asp	Ile 230		Phe	Lys	Gly	Asn 235		Ser	Phe	Arg	Ala 240
	Gly	Ser	Asp	Ala 245		Tyr	Phe	Ala	Gly 250		Glu	Ser	His	Ile 255	
Ala	Leu	Asn	Ala 260		Glu	Gly	His	Ala 265		Val	Phe	His	Asp 270		Leu
Val	Phe	Glu 275	Asn	Leu	Lys	Glu	Arg 280	Lys	Ser	Ala	Glu	Val 285	Leu	Leu	Ile
Asn	Ser 290	Arg	Glu	Asn	Pro	Gly 295	Tyr	Thr	Gly	Ser	Ile 300	Arg	Phe	Leu	Glu
Ala 305	Glu	Ser	Lys	Val	Pro 310	Gln	Cys	Ile	His	Val 315	Gln	Gln	Gly	Ser	Leu 320
Glu	Leu	Leu	Asn	Gly 325	Ala	Thr	Leu	Cys	Ser 330	Tyr	Gly	Phe	Lys	Gln 335	Asp
Ala	Gly	Ala	Lys 340	Leu	Val	Leu	Ala	Ala 345	Gly	Ser	Lys	Leu	Lys 350	Ile	Leu
Asp	Ser	Gly 355	Thr	Pro	Val	Gln	Gly 360	His	Ala	Ile	Ser	Lys 365	Pro	Glu	Ala
Glu	Ile 370	Glu	Ser	Ser	Ser	Glu 375	Pro	Glu	Gly	Ala	His 380	Ser	Leu	Trp	Ile
385					390					395	_		His		400
Ser	Val	Asp	Leu	Ala 405	Ser	Phe	Ser	Ser	Ser 410	Gln	Gln	Glu	Gly	Thr 415	Val
Glu	Ala	Pro	Gln 420	Val	Ile	Val	Pro	Gly 425	Gly	Ser	Tyr	Val	Arg 430	Ser	Gly
		435					440			_		445	Tyr		
	450					455					460		Ser		
Ala 465	Ser	Ser	Asp	Glu	Ala 470	Ser	Ala	Glu	Ile	Ser 475	Asn	Leu	Ser	Val	Ser 480
	Leu	Gln	Ile	His 485		Ala	Thr	Pro	Glu 490		Glu	Glu	Asp	Thr 495	
Gly	His	Met	Gly 500		Trp	Ser	Glu	Ala 505		Ile	Gln	Asp	Gly 510		Leu
Val	Ile	Asn 515		Asn	Pro	Thr	Gly 520		Arg	Leu	Asp	Pro 525	Gln	Lys	Ala
Gly	Ala 530	Leu	Val	Phe	Asn	Ala 535	Leu	Trp	Glu	Glu	Gly 540		Val	Leu	Ser
	Leu	Lys	Asn	Ala		Phe	Ala	His	Asn		Thr	Ala	Gln	Arg	Met
545	Dhe	Aen	ጥኒ፣ኍ	Ser	550 Thr	Δen	Va 1	Trn	Glv	555 Dhe	בומ	Dha	Gly	Glv	560 Dhe
				565					570				Tyr	575	
n.y	1111	neu	580	VI a	JIU	4911	20 u	585	ATG	116	Asp	JLY	590	пур	GIY

Ala Tyr Gly Gly Ala Ser Ala Gly Val Asp Ile Gln Leu Met Glu Asp 600 Phe Val Leu Gly Val Ser Gly Ala Ala Phe Leu Gly Lys Met Asp Ser 620 615 Gln Lys Phe Asp Ala Glu Val Ser Arg Lys Glý Val Val Gly Ser Val 635 630 Tyr Thr Gly Phe Leu Ala Gly Ser Trp Phe Phe Lys Gly Gln Tyr Ser 650 645 Leu Gly Glu Thr Gln Asn Asp Met Lys Thr Arg Tyr Gly Val Leu Gly 665 Glu Ser Ser Ala Ser Trp Thr Ser Arg Gly Val Leu Ala Asp Ala Leu 680 Val Glu Tyr Arg Ser Leu Val Gly Pro Val Arg Pro Thr Phe Tyr Ala 700 695 Leu His Phe Asn Pro Tyr Val Glu Val Ser Tyr Ala Ser Met Lys Phe 715 710 Pro Gly Phe Thr Glu Gln Gly Arg Glu Ala Arg Ser Phe Glu Asp Ala 730 725 Ser Leu Thr Asn Ile Thr Ile Pro Leu Gly Met Lys Phe Glu Leu Ala 745 740 Phe Ile Lys Gly Gln Phe Ser Glu Val Asn Ser Leu Gly Ile Ser Tyr 765 760 Ala Trp Glu Ala Tyr Arg Lys Val Glu Gly Gly Ala Val Gln Leu Leu 775 780 Glu Ala Gly Phe Asp Trp Glu Gly Ala Pro Met Asp Leu Pro Arg Gln 790 795 Glu Leu Arg Val Ala Leu Glu Asn Asn Thr Glu Trp Ser Ser Tyr Phe 805 Ser Thr Val Leu Gly Leu Thr Ala Phe Cys Gly Gly Phe Thr Ser Thr 825 Asp Ser Lys Leu Gly Tyr Glu Ala Asn Thr Gly Leu Arg Leu Ile Phe 840

<210> 193

<211> 778

<212> PRT

<213> Chlamydia

<400> 193

Met His His His His His Gly Leu Ala Ser Cys Val Asp Leu His 10 5 Ala Gly Gly Gln Ser Val Asn Glu Leu Val Tyr Val Gly Pro Gln Ala 20 Val Leu Leu Leu Asp Gln Ile Arg Asp Leu Phe Val Gly Ser Lys Asp Ser Gln Ala Glu Gly Gln Tyr Arg Leu Ile Val Gly Asp Pro Ser Ser 55 Phe Gln Glu Lys Asp Ala Asp Thr Leu Pro Gly Lys Val Glu Gln Ser 70 Thr Leu Phe Ser Val Thr Asn Pro Val Val Phe Gln Gly Val Asp Gln Gln Asp Gln Val Ser Ser Gln Gly Leu Ile Cys Ser Phe Thr Ser Ser 105 Asn Leu Asp Ser Pro Arg Asp Gly Glu Ser Phe Leu Gly Ile Ala Phe 125 120 Val Gly Asp Ser Ser Lys Ala Gly Ile Thr Leu Thr Asp Val Lys Ala 140 135 Ser Leu Ser Gly Ala Ala Leu Tyr Ser Thr Glu Asp Leu Ile Phe Glu

			-												
145					150					155					160
Lys	Ile	Lys	Gly	Gly 165	Leu	Glu	Phe	Ala	Ser 170	Cys	Ser	Ser	Leu	Glu 175	Gln
Gly	Gly	Ala	Cys 180	Ala	Ala	Gln	Ser	Ile 185	Leu	Ile	His	Asp	Cys 190	Gln	Gly
Leu	Gln	Val 195	Lys	His	Cys	Thr	Thr 200	Ala	Val	Asn	Ala	Glu 205	Gly	Ser	Ser
Ala	Asn 210	Asp	His	Leu	Gly	Phe 215	Gly	Gly	Gly	Ala	Phe 220	Phe	Val	Thr	Gly
Ser 225	Leu	Ser	Gly	Glu	Lys 230	Ser	Leu	Tyr	Met	Pro 235	Ala	Gly	Asp	Met	Val 240
Val	Ala	Asn	Суѕ	Asp 245	Gly	Ala	Ile	Ser	Phe 250	Glu	Gly	Asn	Ser	Ala 255	Asn
Phe	Ala	Asn	Gly 260	Gly	Ala	Ile	Ala	Ala 265	Ser	Gly	Lys	Val	Leu 270	Phe	Val
		275	-	-			280		Glu			285			_
•	290					295	_		Ala		300		Ā		
305				_	310	_	٠		Gly	315		_	-	_	320
				325					Gly 330					335	
		_	340		_	_	_	345	Glu				350	_	_
		355	_	_			360		Ser	\		365			
	370	_	_			375	_		Gly	_	380				
385					390				Gln	395					400
_	-	-		405			_	-	Ile 410			-		415	
			420					425	Thr				430	-	
		435					440		Leu			445		_	
	450					455			Ala		460				
465					470	-			Thr	475	-	-			480
				485					Leu 490			_		495	
			500					505	Asn Leu				510		
		515		_			520		Pro			525			
	530					535			Val		540				
545					550					555					560
				565					Gln 570 Ala	_				575	
			580					585	Arg				590		
		595					600		Leu		_	605			
Mec	GTÀ	GIII	GIA	v d I	Ser	GIY	GTA	HIG	₽€U	neu	261	ոհբ	TIIL	val	GIII

620

610

615

```
Leu Ala Gly Asn Gly Ser Val Asp Phe Ser Arg Asn Ile Ala Ser Leu
                           635
           630
Gly Gly Gly Ala Leu Gln Ala Ser Glu Gly Asn Cys Glu Leu Val Asp
                               650
             645
Asn Gly Tyr Val Leu Phe Arg Asp Asn Arg Gly Arg Val Tyr Gly Gly
                            665
Ala Ile Ser Cys Leu Arg Gly Asp Val Val Ile Ser Gly Asn Lys Gly
                        680
Arg Val Glu Phe Lys Asp Asn Ile Ala Thr Arg Leu Tyr Val Glu Glu
                                       700
                    695
Thr Val Glu Lys Val Glu Glu Val Glu Pro Ala Pro Glu Gln Lys Asp
                  710
                                   715
Asn Asn Glu Leu Ser Phe Leu Gly Ser Val Glu Gln Ser Phe Ile Thr
                            730 735
             725
Ala Ala Asn Gln Ala Leu Phe Ala Ser Glu Asp Gly Asp Leu Ser Pro
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Glu Ser Ser Ile Ser Ser Glu Glu Leu Ala Lys Arg Arg Glu Cys Ala
    755 760
Gly Gly Ala Asp Ser Ser Arg Ser Gly Cys
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<400> 194
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Ser Gly Gln Gly Ile Phe Ser Gly Asn Lys Ala Ile Asp Asn Thr Thr
                            25
Glu Gly Ser Ser Ser Lys Ser Asn Val Leu Gly Gly Ala Val Tyr Ala
            40
Lys Thr Leu Phe Asn Leu Asp Ser Gly Ser Ser Arg Arg Thr Val Thr
  50 55
Phe Ser Gly Asn Thr Val Ser Ser Gln Ser Thr Thr Gly Gln Val Ala
                 70
                                    75
Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Ile Ala Thr Pro Val Val
                                90
Phe Ser Lys Asn Ser Ala Thr Asn Asn Ala Asn Asn Ala Thr Asp Thr
                            105
Gln Arg Lys Asp Thr Phe Gly Gly Ala Ile Gly Ala Thr Ser Ala Val
                                           125
                        120
Ser Leu Ser Gly Gly Ala His Phe Leu Glu Asn Val Ala Asp Leu Gly
                     135
                                       140
Ser Ala Ile Gly Leu Val Pro Asp Thr Gln Asn Thr Glu Thr Val Lys
                 150
                                   155
Leu Glu Ser Gly Ser Tyr Tyr Phe Glu Lys Asn Lys Ala Leu Lys Arg
                                170 175
              165
Ala Thr Ile Tyr Ala Pro Val Val Ser Ile Lys Ala Tyr Thr Ala Thr
                            185
Phe Asn Gln Asn Arg Ser Leu Glu Glu Gly Ser Ala Ile Tyr Phe Thr
                                           205
                        200
Lys Glu Ala Ser Ile Glu Ser Leu Gly Ser Val Leu Phe Thr Gly Asn
  210 215
                                       220
Leu Val Thr Pro Thr Leu Ser Thr Thr Thr Glu Gly Thr Pro Ala Thr
                                    235
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Thr	Ser	Gly	Asp	Val 245	Thr	Lys	Tyr	Gly	Ala 250	Ala	Ile	Phe	Gly	Gln 255	Ile
Ala	Ser	Ser	Asn 260	Gly	Ser	Gln	Thr	Asp 265	Asn	Leu	Pro	Leu	Lys 270	Leu	Ile
		275					Phe 280					285			
	290					295	Thr				300				
305					310		Lys			315					320
		_		325		_	Lys		330					335	_
			340				Ser	345					350		
		355	_				Phe 360					365			-
	370					375	Val				380				
385					390		Val Gly			395					400
				405			Asn		410					415	
			420				Glu	425					430		
		435					440 Thr					445			
	450					455	Asn				460				
465	_				470		Gly			475		_			480
		_		485			Thr		490					495	
Ala	Ala	Thr	500 Ala	Thr	Pro	Thr	Thr	505 Thr	Pro	Thr	Ala	Thr	510 Thr	Thr	Thr
Ser	Asn	515 Gln	Val	Ile	Leu	Gly	520 Gly	Glu	Ile	Lys	Leu	525 Ile	Asp	Pro	Asn
Gly	530 Thr	Phe	Phe	Gln	Asn	535 Pro	Ala	Leu	Arg	Ser	540 Asp	Gln	Gln	Ile	Ser
545 Leu	Leu	Val	Leu	Pro	550 Thr	Asp	Ser	Ser	Lys	555 Met	Gln	Ala	Gln	Lys	560 Ile
Val	Leu	Thr	Gly	565 Asp	Ile	Ala	Pro	Gln	570 Lys	Gly	Tyr	Thr	Gly	575 Thr	Leu
Thr	Leu	_	580 Pro	Asp	Gln	Leu	Gln	585 Asn	Gly	Thr	Ile		590 Ala	Leu	Trp
Lys		595 Asp	Ser	Tyr	Arg		600 Trp	Ala	Tyr	Val		605 Arg	Asp	Asn	His
	610 Tyr	Ala	Asn	Ser		615 Leu	Gly	Ser	Gln		620 Ser	Met	Val	Thr	
625 Lys	Gln	Gly	Leu	Leu 645	630 Asn	Asp	Lys	Met	Asn 650	635 Leu	Ala	Arg	Phe	Asp 655	640 Glu
Val	Ser	Tyr	Asn 660		Leu	Trp	Ile	Ser 665		Leu	Gly	Thr	Met 670		Ser
Gln	Val	Gly 675		Pro	Thr	Ser	Glu 680		Phe	Thr	Tyr	Tyr 685		Arg	Gly
Ala	Ser 690		Ala	Leu	Asp	Ala 695	Lys	Pro	Ala	His	Asp 700		Ile	Val	Gly

Ala Ala Phe Ser Lys Met Ile Gly Lys Thr Lys Ser Leu Lys Arg Glu 715 Asn Asn Tyr Thr His Lys Gly Ser Glu Tyr Ser Tyr Gln Ala Ser Val 730 Tyr Gly Gly Lys Pro Phe His Phe Val Ile Asn Lys Lys Thr Glu Lys 745 Ser Leu Pro Leu Leu Gln Gly Val Ile Ser Tyr Gly Tyr Ile Lys 760 His Asp Thr Val Thr His Tyr Pro Thr Ile Arg Glu Arg Asn Gln Gly 775 780 Glu Trp Glu Asp Leu Gly Trp Leu Thr Ala Leu Arg Val Ser Ser Val 790 795 Leu Arg Thr Pro Ala Gln Gly Asp Thr Lys Arg Ile Thr Val Tyr Gly 805 810 Glu Leu Glu Tyr Ser Ser Ile Arg Gln Lys Gln Phe Thr Glu Thr Glu 825 Tyr Asp Pro Arg Tyr Phe Asp Asn Cys Thr Tyr Arg Asn Leu Ala Ile 840 Pro Met Gly Leu Ala Phe Glu Gly Glu Leu Ser Gly Asn Asp Ile Leu 855 Met Tyr Asn Arg Phe Ser Val Ala Tyr Met Pro Ser Ile Tyr Arg Asn 870 875 Ser Pro Thr Cys Lys Tyr Gln Val Leu Ser Ser Gly Glu Gly Glu 890 885 Ile Ile Cys Gly Val Pro Thr Arg Asn Ser Ala Arg Gly Glu Tyr Ser 905 Thr Gln Leu Tyr Pro Gly Pro Leu Trp Thr Leu Tyr Gly Ser Tyr Thr 925 Ile Glu Ala Asp Ala His Thr Leu Ala His Met Met Asn Cys Gly Ala 935 Arg Met Thr Phe 945 <210> 195 <211> 821 <212> PRT <213> Chlamydia <400> 195 Met His His His His His Glu Ala Ser Ser Ile Gln Asp Gln Ile 5 10 Lys Asn Thr Asp Cys Asn Val Ser Lys Val Gly Tyr Ser Thr Ser Gln 25 Ala Phe Thr Asp Met Met Leu Ala Asp Asn Thr Glu Tyr Arg Ala Ala 40 Asp Ser Val Ser Phe Tyr Asp Phe Ser Thr Ser Ser Gly Leu Pro Arg Lys His Leu Ser Ser Ser Ser Glu Ala Ser Pro Thr Thr Glu Gly Val 70 75 Ser Ser Ser Ser Gly Glu Asn Thr Glu Asn Ser Gln Asp Ser Ala 85 90 Pro Ser Ser Gly Glu Thr Asp Lys Lys Thr Glu Glu Glu Leu Asp Asn 105 Gly Gly Ile Ile Tyr Ala Arg Glu Lys Leu Thr Ile Ser Glu Ser Gln 120 125 Asp Ser Leu Ser Asn Pro Ser Ile Glu Leu His Asp Asn Ser Phe Phe 135 Phe Gly Glu Gly Glu Val Ile Phe Asp His Arg Val Ala Leu Lys Asn

145					150					155					160
Gly	Gly	Ala	Ile	Tyr	Gly	Glu	Lys	Glu	Val	Val	Phe	Glu	Asn	Ile	Lys
				165					170					175	
Ser	Leu	Leu	Val	Glu	Val	Asn	Ile	Ser	Val	Glu	Lys	Gly	Gly	Ser	Val
			180					185			•	•	190		
Tvr	Δla	Lvs		Ara	Val	Ser	I.e.11		Δen	Va1	Thr	Glu		Thr	Phe
- / -	7114	195	014			001	200	014	11011	•		205			1110
C	00=		~1	~1	~1	Gln		a 1	~1	~1	71 0	-	0	a 1	a 1-
261		ASII	GIY	GIY	GIU		GIY	GIY	GIY	GIA		ıyı	ser	GIU	GIII
.	210		-1.			215	_	_	1		220	~1	<i>~</i> 1		
		Leu	TTE	ser	_	Cys	Asn	Asn	vaı		Pne	Gin	GLY	Asn	
225				_	230		_			235				_	240
Ala	Gly	Ala	Thr	Ala	Val	Lys	Gln	Cys	Leu	Asp	Glu	Glu	Met	Tle	Val
				245					250					255	
Leu	Leu	Thr	Glu	Cys	Val	Asp	Ser	Leu	Ser	Glu	Asp	Thr	Leu	Asp	Ser
			260					265					270		
Thr	Pro	Glu	Thr	Glu	Gln	Thr	Lys	Ser	Asn	Gly	Asn	Gln	Asp	Gly	Ser
		275					280					285			
Ser	Glu	Thr	Lys	Asp	Thr	Gln	Val	Ser	Glu	Ser	Pro	Glu	Ser	Thr	Pro
	290		-			295					300				
Ser	Pro	Asp	Asp	Val	Leu	Gly	Lvs	Glv	Glv	Glv	Ile	Tvr	Thr	Glu	Lvs
305					310	2	_,_	2	2	315		- 2 -			320
	Leu	Thr	Tle	Thr		Ile	Thr	Glv	Thr		Asp	Phe	Val	Ser	
				325	017			017	330					335	••••
T۱۵	Δla	Thr	Acn		Glv	Ala	Glv	Val		Thr	Lare	Glu	Aen		Sar
116	AIG	1111	340	361	GIY	AIG	GLY	345	FIIC	1111	Lys	Giu	350	Deu	261
Cvc	Th.~	Zan		λan	C0~	Leu	cln		Low	Tara) an	602		C1	C1 n
Cys	1111		IIII	ASII	Ser	Бец		Pne	Leu	ьуѕ	ASII		АІА	GLY	GIII
***	a 1	355	a1	n 1 -	m	17-1	360	~1 ·-	mb		0	365	m\		m)
HIS	_	GIY	GIY	АТА	Tyr	Val	Thr	GIn	inr	met		vaı	Thr	Asn	inr
m)	370	~1		-1.	m1	375	_		_	,	380	~1		- 1	-1
	Ser	GIu	Ser	He		Thr	Pro	Pro	Leu		Gly	Glu	Val	He	
385	_				390	_		_	_	395	_				400
Ser	Glu	Asn	Thr		Lys	Gly	His	Gly	_	Gly	Ile	Cys	Thr		Lys
				405					410					415	
Leu	Ser	Leu	Ser	Asn	Leu	Lys	Thr	Val	Thr	Leu	Thr	Lys	Asn'	Ser	Ala
			420					425					430		
Lys	Glu	Ser	Gly	Gly	Ala	Ile	Phe	Thr	Asp	Leu	Ala	Ser	Ile	Pro	Thr
		435					440					445			
Thr	Asp	Thr	Pro	Glu	Ser	Ser	Thr	Pro	Ser	Ser	Ser	Ser	Pro	Ala	Ser
	450					455					460				
Thr	Pro	Glu	Val	Val	Ala	Ser	Ala	Lys	Ile	Asn	Arg	Phe	Phe	Ala	Ser
465					470			•		475	_				480
Thr	Ala	Glu	Pro	Ala	Ala	Pro	Ser	Leu	Thr	Glu	Ala	Glu	Ser	Asp	Gln
			_	485										495	
Thr	Asp	Gln	Thr		Thr	Ser	Asp	Thr	Δsn	Ser	Asp	Tle			
			500	0		002		505		501			510	, 41	-
Tla	Glu	λen		Lan	Acn	Val	λla		Acn	Gln	λen	Thr		NΙα	Luc
116	GIU	515	116	Бец	ASII	Vai	520	116	ASII	GIII	ASII	525	Ser	AIG	цуз
T	C1		71-	т1 ^	TPs #36	C1		T 110	77.	T	T 011		7~~	т1о	7.00
гуs		GTÅ	Ald	TIE	I Y L	Gly	rys	гÀг	AId	ьуѕ		ser	Arg	116	ASII
_	530	~ 1	-	_	~ 3	535			~1		540	~7	~1.	~ 3	.
	Leu	GIu	Leu	Ser	-	Asn	Ser	Ser	GIn	_	Val	GIA	GIY	GIY	
545					550	_	_		_	555	_				560
Cys	Leu	Thr	Glu	Ser	Val	Glu	Phe	Asp		Ile	Gly	Ser	Leu		Ser
				565					570					575	
His	Tyr	Asn	Ser	Ala	Ala	Lys	Glu	Gly	Gly	Val	Ile	His	Ser	Lys	Thr
			580					585					590		
Val	Thr	Leu	Ser	Asn	Leu	Lys	Ser	Thr	Phe	Thr	Phe	Ala	Asp	Asn	Thr
		595					600					605	•		
Val	Lys		Ile	Val	Glu	Ser	Thr	Pro	Glu	Ala	Pro	Glu	Glu	Ile	Pro
	-														

104

610 615 Pro Val Glu Glu Glu Ser Thr Ala Thr Glu Asn Pro Asn Ser Asn 630 635 Thr Glu Gly Ser Ser Ala Asn Thr Asn Leu Glu Gly Ser Gln Gly Asp 645 650 Thr Ala Asp Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr 665 670 Ser Asp Thr Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr 680 Gln Ser Asn Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser 695 700 Asn Glu Asn Thr Asp Glu Ser Ser Asp Ser His Thr Glu Glu Ile Thr 715 Asp Glu Ser Val Ser Ser Ser Ser Lys Ser Gly Ser Ser Thr Pro Gln 725 Asp Gly Gly Ala Ala Ser Ser Gly Ala Pro Ser Gly Asp Gln Ser Ile 740 745 Ser Ala Asn Ala Cys Leu Ala Lys Ser Tyr Ala Ala Ser Thr Asp Ser 760 Ser Pro Val Ser Asn Ser Ser Gly Ser Asp Val Thr Ala Ser Ser Asp 775 780 Asn Pro Asp Ser Ser Ser Ser Gly Asp Ser Ala Gly Asp Ser Glu Gly 795 Pro Thr Glu Pro Glu Ala Gly Ser Thr Thr Glu Thr Pro Thr Leu Ile 805 Gly Gly Gly Ala Ile 820 <210> 196 <211> 525 <212> PRT <213> Chlamydia <400> 196 Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu 5 10 Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala 25 Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val 55 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr 70 75 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr 85 90 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser 105 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr 120 125 Leu Ala Glu Gly Pro Pro Ala Glu Phe Pro Leu Val Pro Arg Gly Ser 135 140 Pro Leu Pro Val Gly Asn Pro Ala Glu Pro Ser Leu Leu Ile Asp Gly 150 155 Thr Met Trp Glu Gly Ala Ser Gly Asp Pro Cys Asp Pro Cys Ala Thr 165 170 Trp Cys Asp Ala Ile Ser Ile Arg Ala Gly Tyr Tyr Gly Asp Tyr Val

185

105

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Phe Asp Arg Val Leu Lys Val Asp Val Asn Lys Thr Phe Ser Gly Met
                         200
Ala Ala Thr Pro Thr Gln Ala Ile Gly Asn Ala Ser Asn Thr Asn Gln
                    215
                                       220
Pro Glu Ala Asn Gly Arg Pro Asn Ile Ala Tyr Gly Arg His Met Gln
                  230
                                    235
Asp Ala Glu Trp Phe Ser Asn Ala Ala Phe Leu Ala Leu Asn Ile Trp
              245
                      250
Asp Arg Phe Asp Ile Phe Cys Thr Leu Gly Ala Ser Asn Gly Tyr Phe
                             265
Lys Ala Ser Ser Ala Ala Phe Asn Leu Val Gly Leu Ile Gly Phe Ser
                         280
Ala Ala Ser Ser Ile Ser Thr Asp Leu Pro Met Gln Leu Pro Asn Val
                     295
                                        300
Gly Ile Thr Gln Gly Val Val Glu Phe Tyr Thr Asp Thr Ser Phe Ser
                  310
                                     315
Trp Ser Val Gly Ala Arg Gly Ala Leu Trp Glu Cys Gly Cys Ala Thr
              325
                                330
Leu Gly Ala Glu Phe Gln Tyr Ala Gln Ser Asn Pro Lys Ile Glu Met
                            345
Leu Asn Val Thr Ser Ser Pro Ala Gln Phe Val Ile His Lys Pro Arg
      355 . 360
Gly Tyr Lys Gly Ala Ser Ser Asn Phe Pro Leu Pro Ile Thr Ala Gly
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Thr Thr Glu Ala Thr Asp Thr Lys Ser Ala Thr Ile Lys Tyr His Glu
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Trp Gln Val Gly Leu Ala Leu Ser Tyr Arg Leu Asn Met Leu Val Pro
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Tyr Ile Gly Val Asn Trp Ser Arg Ala Thr Phe Asp Ala Asp Thr Ile
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Arg Ile Ala Gln Pro Lys Leu Lys Ser Glu Ile Leu Asn Ile Thr Thr
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Trp Asn Pro Ser Leu Ile Gly Ser Thr Thr Ala Leu Pro Asn Asn Ser
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114

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118

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897

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120

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Th	r '		Leu	Ala	Thr	Phe 150		Ala	Ile	Arg	Pro 155	Ile	Leu	Phe	Val	Asn 160		
Lу	s l	Met	Leu	Ala	Lys 165	Pro	Phe	Leu	Ser	Ser 170		Thr	Lys	Ala	Asn 175			
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Gl	u (Cys	Val	Ala 260	Asp	Val	Phe	Lys	Leu 265	Val	Pro	Leu	Pro	Ile 270	Thr	Met		
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Cys Pro Thr Glu Leu His Ala Phe Gln Asp Arg Leu Val Asp Phe Glu

Glu His Gly Ala Val Val Leu Gly Cys Ser Val Asp Asp Ile Glu Thr

His Ser Arg Trp Leu Thr Val Ala Arg Asp Ala Gly Gly Ile Glu Gly

Thr Glu Tyr Pro Leu Leu Ala Asp Pro Ser Phe Lys Ile Ser Glu Ala 105

Phe Gly Val Leu Asn Pro Glu Gly Ser Leu Ala Leu Arg Ala Thr Phe 120

Leu Ile Asp Lys His Gly Val Ile Arg His Ala Val Ile Asn Asp Leu 135

Pro Leu Gly Arg Ser Ile Asp Glu Glu Leu Arg Ile Leu Asp Ser Leu

PCT/US00/32919 WO 01/40474

130

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Gln Thr Met Asp 195

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Val Gly Ala Leu Val Val Val Ala Gly Val Leu Ala Leu Val Leu Cys

Ala Ser Asn Val Ile Phe Thr Val Ile Gly Ile Pro Ala Leu Ile Ile

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Arg Asn Leu Ser Glu Glu Lys Asp Ala Leu Ala Ser Val Ser Phe Ile

Asn Lys Met Phe Leu Arg Gly Leu Thr Asp Asp Leu Gln Ala Leu Glu

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35 40 45

Pro Leu Pro Ser Arg Tyr Gln Leu Gln Asn Met Asp Val Glu Ala Gly
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Phe Arg Glu Ala Val Tyr Ala Ser Phe Val Ala Gly Met Tyr Asn Tyr 65 70 75 80

Val Val Thr Gln Pro Gln Glu Arg Ile Pro Asn Ser Gln Gln Val Glu
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Val Cys Lys Asn Ala Leu Gln Asp Leu Ser Phe Leu Glu His Leu Leu 50 55 60

Gln Val Lys Tyr Ala Pro Lys Thr Trp Lys Glu Gln Tyr Leu Gly Trp
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Asp Leu Val Gln Ser Ser Val Ser Ala Gln Gln Lys Leu Arg Thr Gln
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Glu Asn Pro Ser Thr Ser Phe Cys Gln Gln Val Leu Ala Asp Phe Ile 100 105 110

Gly Gly Leu Asn Asp Phe His Ala Gly Val Thr Phe Phe Ala Ile Glu 115 120 125

Ser Ala Tyr Leu Pro Tyr Thr Val Gln Lys Ser Ser Asp Gly Arg Phe 130 135 140

Tyr Phe Val Asp Ile Met Thr Phe Ser Ser Glu Ile Arg Val Gly Asp Glu Leu Leu Glu Val Asp Gly Ala Pro Val Gln Asp Val Leu Ala Thr Leu Tyr Gly Ser Asn His Lys Gly Thr Ala Ala Glu Glu Ser Ala Ala 185 Leu Arg Thr Leu Phe Ser Arg Met Ala Ser Leu Gly His Lys Val Pro Ser Gly Arg Thr Thr Leu Lys Ile Arg Arg Pro Phe Gly Thr Thr Arg Glu Val Arg Val Lys Trp Arg Tyr Val Pro Glu Gly Val Gly Asp Leu 230 Ala Thr Ile Ala Pro Ser Ile Arg Ala Pro Gln Leu Gln Lys Ser Met Arg Ser Phe Phe Pro Lys Lys Asp Asp Ala Phe His Arg Ser Ser Ser Leu Phe Tyr Ser Pro Met Val Pro His Phe Trp Ala Glu Leu Arg Asn His Tyr Ala Thr Ser Gly Leu Lys Ser Gly Tyr Asn Ile Gly Ser Thr Asp Gly Phe Leu Pro Val Ile Gly Pro Val Ile Trp Glu Ser Glu Gly 310 Leu Phe Arg Ala Tyr Ile Ser Ser Val Thr Asp Gly Asp Gly Lys Ser 330 His Lys Val Gly Phe Leu Arg Ile Pro Thr Tyr Ser Trp Gln Asp Met Glu Asp Phe Asp Pro Ser Gly Pro Pro Pro Trp Glu Glu Phe Ala Lys 360 Ile Ile Gln Val Phe Ser Ser Asn Thr Glu Ala Leu Ile Ile Asp Gln 375 Thr Asn Asn Pro Gly Gly Ser Val Leu Tyr Leu Tyr Ala Leu Leu Ser 395 390 Met Leu Thr Asp Arg Pro Leu Glu Leu Pro Lys His Arg Met Ile Leu Thr Gln Asp Glu Val Val Asp Ala Leu Asp Trp Leu Thr Leu Leu Glu 425 Asn Val Asp Thr Asn Val Glu Ser Arg Leu Ala Leu Gly Asp Asn Met 440

Glu Gly Tyr Thr Val Asp Leu Gln Val Ala Glu Tyr Leu Lys Ser Phe

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Thr Pro Ile Pro Leu Phe Gly Phe 485

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Val Ser Ser Val Leu Asp Asn Val Pro Leu Val Ala Ala Thr Ile Gly

Met Tyr Asp Leu Pro Met Asn Asp Pro Leu Trp Lys Leu Ile Ala Tyr

Thr Ala Gly Thr Gly Gly Ser Ile Leu Ile Ile Gly Ser Ala Ala Gly

Val Ala Tyr Met Gly Met Glu Lys Val Ser Phe Gly Trp Tyr Val Lys

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Tyr Phe Leu Met Glu Asn Cys Val Asn Leu Phe Val 135

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<212> PRT

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<400> 299

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Ala Ala Gly Ala Leu Lys Ser Ser Asn Asn Ser Gly Arg Ile Ser Leu 55 Leu Leu Asp Asp Val Asp Asn Glu Met Ala Ala Ile Ala Met Gln Gly Phe Arg Ser Met Ile Glu Gln Phe Asn Val Asn Asn Pro Ala Thr Ala Lys Glu Leu Gln Ala Met Glu Ala Gln Leu Thr Ala Met Ser Asp Gln 105 Leu Val Gly Ala Asp Gly Glu Leu Pro Ala Glu Ile Gln Ala Ile Lys 120 Asp Ala Leu Ala Gln Ala Leu Lys Gln Pro Ser Ala Asp Gly Leu Ala 135 Thr Ala Met Gly Gln Val Ala Phe Ala Ala Ala Lys Val Gly Gly Ser Ala Gly Thr Ala Gly Thr Val Gln Met Asn Val Lys Gln Leu Tyr 170 Lys Thr Ala Phe Ser Ser Thr Ser Ser Ser Tyr Ala Ala Ala Leu Ser Asp Gly Tyr Ser Ala Tyr Lys Thr Leu Asn Ser Leu Tyr Ser Glu 200 Ser Arg Ser Gly Val Gln Ser Ala Ile Ser Gln Thr Ala Asn Pro Ala 215 Leu Ser Arg Ser Val Ser Arg Ser Gly Ile Glu Ser Gln Gly Arg Ser 235 Ala Asp Ala Ser Gln Arg Ala Ala Glu Thr Ile Val Arg Asp Ser Gln 250 Thr Leu Gly Asp Val Tyr Ser Arg Leu Gln Val Leu Asp Ser Leu Met Ser Thr Ile Val Ser Asn Pro Gln Ala Asn Gln Glu Glu Ile Met Gln 280 Lys Leu Thr Ala Ser Ile Ser Lys Ala Pro Gln Phe Gly Tyr Pro Ala 295 Val Gln Asn Ser Val Asp Ser Leu Gln Lys Phe Ala Ala Gln Leu Glu 310 Arg Glu Phe Val Asp Gly Glu Arg Ser Leu Ala Glu Ser Gln Glu Asn 330 Ala Phe Arg Lys Gln Pro Ala Phe Ile Gln Gln Val Leu Val Asn Ile 345

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Pro Ser Ala Gly Ile Asp Glu Ser Asn Thr Asp Gln Pro Phe Val Leu

Tyr Pro Lys Asp Ile Leu Gly Ser Cys Asn Arg Ile Gly Glu Trp Leu

Arg Asn Tyr Phe Arg Val Lys Glu Leu Gly Val Ile Ile Thr Asp Ser

His Thr Thr Pro Met Arg Arg Gly Val Leu Gly Ile Gly Leu Cys Trp

Tyr Gly Phe Ser Pro Leu His Asn Tyr Ile Gly Ser Leu Asp Cys Phe 120

Gly Arg Pro Leu Gln Met Thr Gln Ser Asn Leu Val Asp Ala Leu Ala

Val Ala Ala Val Val Cys Met Gly Glu Gly Asn Glu Gln Thr Pro Leu

Ala Val Ile Glu Gln Ala Pro Asn Met Val Tyr His Ser Tyr Pro Thr 170

Ser Arg Glu Glu Tyr Cys Ser Leu Arg Ile Asp Glu Thr Glu Asp Leu

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Asp Glu Ile Gln Gln Met Ile Arg Asp Ala Glu Leu His Lys Glu Glu
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Asp Lys Gln Arg Lys Glu Ala Ser Asp Val Lys Asn Glu Ala Asp Gly 65 70 75 80

Met Ile Phe Arg Ala Glu Lys Ala Val Lys Asp Tyr His Asp Lys Ile 85 90 95

Pro Ala Glu Leu Val Lys Glu Ile Glu Glu His Ile Glu Lys Val Arg 100 105 110

Gln Ala Ile Lys Glu Asp Ala Ser Thr Thr Ala Ile Lys Ala Ala Ser 115 120 125

Asp Glu Leu Ser Thr Arg Met Gln Lys Ile Gly Glu Ala Met Gln Ala 130 135 140

Gln Ser Ala Ser Ala Ala Ala Ser Ser Ala Ala Asn Ala Gln Gly Gly 145 150 155 160

Pro Asn Ile Asn Ser Glu Asp Leu Lys Lys His Ser Phe Ser Thr Arg 165 170 175

Pro Pro Ala Gly Gly Ser Ala 180

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Cys Pro Thr Val Arg Phe Asp Gln Thr Val Asp Val Ser Val Lys Leu 35 40 45

Gly Ile Asp Pro Arg Lys Ser Asp Gln Gln Ile Arg Gly Ser Val Ser 50 60

Leu Pro His Gly Thr Gly Lys Val Leu Arg Ile Leu Val Phe Ala Ala 65 70 75 80

Gly Asp Lys Ala Ala Glu Ala Ile Glu Ala Gly Ala Asp Phe Val Gly 85 90 95

Ser Asp Asp Leu Val Glu Lys Ile Lys Gly Gly Trp Val Asp Phe Asp 100 105 110

Val Ala Val Ala Thr Pro Asp Met Met Arg Glu Val Gly Lys Leu Gly

Lys Val Leu Gly Pro Arg Asn Leu Met Pro Thr Pro Lys Ala Gly Thr 130 140

Val Thr Thr Asp Val Val Lys Thr Ile Ala Glu Leu Arg Lys Gly Lys
145 150 155 160

Ile Glu Phe Lys Ala Asp Arg Ala Gly Val Cys Asn Val Gly Val Ala 165 170 175

Lys Leu Ser Phe Asp Ser Ala Gln Ile Lys Glu Asn Val Glu Ala Leu

Cys Ala Ala Leu Val Lys Ala Lys Pro Ala Thr Ala Lys Gly Gln Tyr 195 200 205

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Glu Pro Leu Lys Asp Gln Gln Ile Ile Leu Gly Thr Thr Ser Thr Pro
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Val Ala Ala Lys Met Thr Ala Ser Asp Gly Ile Ser Leu Thr Val Ser 65 70 75 80

Asn Asn Pro Ser Thr Asn Ala Ser Ile Thr Ile Gly Leu Asp Ala Glu 85 90 95

Lys Ala Tyr Gln Leu Ile Leu Glu Lys Leu Gly Asp Gln Ile Leu Gly 100 105 110

Gly Ile Ala Asp Thr Ile Val Asp Ser Thr Val Gln Asp Ile Leu Asp 115 120 125

Lys Ile Thr Thr Asp Pro Ser Leu Gly Leu Leu Lys Ala Phe Asn Asn 130 135 140

Phe Pro Ile Thr Asn Lys Ile Gln Cys Asn Gly Leu Phe Thr Pro Arg 145 150 155 160

Asn Ile Glu Thr Leu Leu Gly Gly Thr Glu Ile Gly Lys Phe Thr Val 165 170 175

Thr Pro Lys Ser Ser Gly Ser Met Phe Leu Val Ser Ala Asp Ile Ile 180 185 190

Ala Ser Arg Met Glu Gly Gly Val Leu Ala Leu Val Arg Glu Gly
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Asn Lys Met Ala Arg Val Val Asn Lys Thr Lys Gly Met Asp Lys Thr 35 40 45

Val Lys Val Ala Lys Ser Ala Ala Glu Leu Thr Ala Asn Ile Leu Glu 50 55 60

Gln Ala Gly Gly Ala Gly Ser Ser Ala His Ile Thr Ala Ser Gln Val
65 70 75 80

Ser Lys Gly Leu Gly Asp Thr Arg Thr Val Val Ala Leu Gly Asn Ala 85 90 95

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420

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540

600

660

720

780

840 900

960

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Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala
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Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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                             120
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                    150
 Ile His Ala His Tyr Asp Gln Arg Gln Leu Ser Gln Ser Pro Asn Thr
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                                     170
 Asn Phe Leu Val His His Pro Tyr Leu Thr Leu Ile Pro Lys Phe Leu
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Ser	Ala	Ser		His	Phe	Lys	His		Asn	Ile	Asn	Gly		Gly	Ala
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Ile		Glv	Asp	Phe	Ala		Ser	Arq	Ile	Leu		Leu	Asn	Asn	Gln
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Phe	Lys		Asn	Thr	Thr	Ile		Asn	Gly	Gly	Ala		Tyr	Thr	Ser
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465	C1 5	7 an	۵۱.	7.00	470	T1.	7	Dho	C1	475	7 ~~	mb w	77.	T	480
GIU	GIII	ASII	GIII	485	1111	116	Arg	Pile	490	Lys	ASII	1111	на	495	Giu
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	` •	_,	500	_	_	_		505		_	_	3	510		
Thr	He	7nr 515	Pne	ser	Asp	Asn	520	Ala	GIY	Asp	Leu	525	GIŸ	Gly	Ala
Ile		Leu	Glu	${\tt Gly}$	Lys	-	Pro	Ser	Leu	Thr		Ile	Ala	His	Ser
al.	530	77 -	33 -	DL -	C	535	3	m)		¥	540	~1.	m\	•	T
545	ASN	11e	Ala	Pne	550	GIY	ASN	Thr	мес	Leu 555	HIS	116	inr	rys	ьуs 560
	Ser	Leu	Asp	Arg		Asn	Ser	Ile	Leu	Ile	Lys	Glu	Ala	Pro	
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Pro	Glu		Glu	Thr	Pro	Phe		Ser	Pro	Lys					•
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<213> Chlamydia trachomatis

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144

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          500
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Gly Lys Trp Asp Thr Phe Gly Met Arg Gly Thr Leu Gly Ala Ser Tyr
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Thr Phe Leu Glu Tyr Asp Gln Thr Met Arg Val Phe Ser Phe Ala Asn
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Asn Pro Arg Ser Phe Ser Lys Thr Lys Leu Leu Asn Ile Ala Ile Pro
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Ile Gly Ile Gly Tyr Glu Phe Cys Leu Gly Asn Ser Ser Phe Ala Leu
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Leu Gly Lys Gly Ser Ile Gly Tyr Ser Arg Asp Ile Lys Arg Glu Asn
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Asn Gly Cys Ser Val Pro Thr Ser Ala His Thr Leu Ala Asn Gln Leu
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Phe Phe Tyr Leu Ser Thr Ile Ser Ile Leu Gln Ala Asn Glu Thr Asp

170

165

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		195	Pro		Ser		200					205			
	210	Gln			Gly	215					220				
225	Thr				Ser 230					235					240
Gly				245	Val				250					255	
			260		Asn			265					2/0		
		275			Gly		280					285			
	200				Ile	295					300				
205					Asn 310					315					320
				325	Arg				330					333	
			340		Ile			345					350		
		355			Leu		360					365			
	270				Glu	375					380				
205					Ser 390					395					400
				405	Gly				410					413	
			420		Lys			425					430		
		435			Ser		440					445			Phe
	450	١				455					460				Tyr
165					470 Asn					475					400
				485	5				490	}				493	Asn
			500)				505	5				510	,	Val
		515	5				520)				523	•		Ile
	536	n .				535	5				54()			Lys
E 4 6	=				550)				555	•				560 Lys
				569	5				570)				5/5	lle
			581	0				58!	5				590	,	Tyr
		59	5				600	0				60:	>		Asn
	61	0				61	5				620	J			Ile
Ty:		е гу	s GI	u Ali	630		_ 111.	- ne	~ ny:	63	5				640

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1860

1920

1980

2040

2100 2148

ggttcttggg tcaccacaaa taccccatta gctaaacatt ccttttatgg gagaggttct cactecetea aattttetea tetgaaaeta tttgetaaet ateaageaga agtggetaet tecaetgtet cacactacat caatgeagga ggagetetgg tettttaa <210> 321 <211> 715 <212> PRT <213> Chlamydia trachomatis Met His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu 10 Ser Gln Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala 25 20 Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala 40 35 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val 60 55 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr 75 70 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr 90 85 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser 105 110 100 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr 125 120 Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp 130 135 140 Arg Pro Leu Asp Ile Arg Thr Leu Met Gly Lys Glu His Asn Tyr Ile 150 155 Lys Glu Ala Pro Thr Thr Leu Lys Phe Gly Thr Leu Ala Ile Glu Asp 170 165 Asp Ala Glu Leu Glu Ile Phe Asn Ile Pro Phe Thr Gln Asn Pro Thr 185 Ser Leu Leu Ala Leu Gly Ser Gly Ala Thr Leu Thr Val Gly Lys His 205 200 Gly Lys Leu Asn Ile Thr Asn Leu Gly Val Ile Leu Pro Ile Ile Leu 220 215 Lys Glu Gly Lys Ser Pro Pro Cys Ile Arg Val Asn Pro Gln Asp Met 230 235 Thr Gln Asn Thr Gly Thr Gly Gln Thr Pro Ser Ser Thr Ser Ser Ile 250 255 245 Ser Thr Pro Met Ile Ile Phe Asn Gly Arg Leu Ser Ile Val Asp Glu 265 Asn Tyr Glu Ser Val Tyr Asp Ser Met Asp Leu Ser Arg Gly Lys Ala 280 285 Glu Gln Leu Ile Leu Ser Ile Glu Thr Thr Asn Asp Gly Gln Leu Asp 300 295 Ser Asn Trp Gln Ser Ser Leu Asn Thr Ser Leu Leu Ser Pro Pro His 310 315 Tyr Gly Tyr Gln Gly Leu Trp Thr Pro Asn Trp Ile Thr Thr Tyr 330 325 Thr Ile Thr Leu Asn Asn Asn Ser Ser Ala Pro Thr Ser Ala Thr Ser 345 350 340 Ile Ala Glu Gln Lys Lys Thr Ser Glu Thr Phe Thr Pro Ser Asn Thr 360 365 355 Thr Thr Ala Ser Ile Pro Asn Ile Lys Ala Ser Ala Gly Ser Gly Ser 375

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His Gly Tyr Ser Ser Ala Ser Lys Gly Tyr Thr Val Ser Ser Gln Ala
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Ser Gly Ala His Gly His Lys Phe Leu Leu Ser Phe Ser Gln Ser Ser
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Ser Ile Met Leu Thr Pro Phe Ala Gln Ala Leu Phe Ser Arg Thr Glu
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Ile Gln Asn Asn Gly Ser Trp Val Thr Thr Asn Thr Pro Leu Ala Lys
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                                                                      1800
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                                                                      1896
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                             40
 Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
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 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
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Leu	Ala 130	Glu	Gly	Pro	Pro	Ala 135	Glu	Phe	Cys	Arg	Tyr 140	Pro	Ser	His	Trp
145		Leu			150					155					160
Ala	Cys	Leu	Cys	Ser 165	Tyr	Ser	Tyr	Gly	Phe 170	Ala	Ser	Ser	Pro	Gln 175	Val
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	_	Asp 195	-				200		-		_	205			_
	210	Ile				215					220		_		
225		Leu			230					235					240
		Ile Phe		245					250					255	
		Thr	260	-				265	_		_	_	270		
		275					280					285		_	
	290	Val				295					300				
305	Pro	Pro	АТА	Pro	310	Pro	Ala	Pro	Ala	315	ser	ser	ser	Leu	320
		Val	•	325			_	_	330					335	
		Glu	340					345					350		
		Asn 355	٠.				360					365			
	370	Gly				375		V			380				
385		Lys		_	390				_	395					400
		Val		405					410					415	
		Phe	420	_	_			425	_	_			430		
		Thr 435		_	_		440					445			_
	450	Cys				455					460				
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		Thr	500					505					510		
		Ser 515					520					525			
	530	Gly				535					540				
Gly 545	Asn	Leu	Thr	Leu	Ser 550	Ala	Ser	Asp	Gly	Asp 555	Ile	Val	Phe	Ser	Gly 560

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Ser	Gly 290		Thr	Leu	Asp	Leu 295		Met	Glu	Asn	Glu 300		Glu	Val	Tyr
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Gly Phe Leu Ala Arg Tyr Gly Gly Phe Gln Ala Thr Ala Ala Ser His
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Tyr Glu Asn Gly Ser Ile Phe Gly Val Ala Phe Gly Gln Leu Tyr Gly
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Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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<213> Chlamydia trachomatis

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gctaataaac gtggagcgat ttactctcct agcgtttcca ttaaagggaa taatattacc
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Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
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Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
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Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
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                           120
Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp
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Arg Pro Leu Asp Gln Leu Asn Ile Ser Glu Asn Ser Ala Thr Glu Ile
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Gly Gly Gly Ile Cys Cys Lys Glu Ser Leu Glu Leu Asp Ala Leu Val
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Ser Leu Ser Val Thr Glu Asn Leu Val Gly Lys Glu Gly Gly Leu
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His Ala Lys Thr Val Asn Ile Ser Asn Leu Lys Ser Gly Phe Ser Phe
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                              265
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Phe Cys Ser Ile Ala Gly Tyr Val Lys Leu Ser Leu Gln Ala Ala Lys
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        580 585
Thr Gly Ser Thr Gln Asn Val Tyr Glu Thr Leu Asp Ile Asn Lys Glu
     595 600 605
Glu Asn Ser Asn Pro Tyr Thr Gly Thr Ile Val Phe Ser Ser Glu Leu
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                         620
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Gln 65	Arg	Val	Val	Gly	Ser 70	Ala	Pro	Ala	Ala	Ser 75	Leu	Gly	Ile	Ser	Thr 80
	Asp	Val	Ile	Thr 85	Ala	Val	Asp	Gly	Ala 90	Pro	Ile	Asn	Ser	Ala 95	
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Val	Thr	Trp 115		Thr	Lys	Ser	Gly 120		Thr	Arg	Thr	Gly 125		Val	Thr
Leu	Ala 130	Glu	Gly	Pro	Pro	Ala 135	Glu	Phe	Cys	Arg	Tyr 140	Pro	Ser	His	Trp
Arg 145	Pro	Leu	Gly	Thr	Ile 150	Val	Phe	Ser	Ser	Glu 155	Leu	His	Glu	Asn	Lys 160
Ser	Tyr	Ile	Pro	Gln 165	Asn	Ala	Ile	Leu	His 170	Asn	Gly	Thr	Leu	Val 175	Leu .
Lys	Glu	Lys	Thr 180	Glu	Leu	His	Val	Val 185	Ser	Phe	Glu	Gln	Lys 190	Glu	Gly
Ser	Lys	Leu 195	Ile	Met	Glu	Pro	Gly 200	Ala	Val	Leu	Ser	Asn 205	Gln	Asn	Ile
Ala	Asn 210	Gly	Ala	Leu	Ala	Ile 215	Asn	Gly	Leu	Thr	Ile 220	Asp	Leu	Ser	Ser
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				245		Ser			250					255	
			260			Lys		265					270		_
		275				Thr	280					285			
	290					Asp 295					300				
305		_		_	310	Asp _	-			315					320
				325		Tyr			330					335	
			340			Gly		345					350		
		355				Arg	360					365			
	370					Asn 375					380				
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				405		Asp			410	_				415	
			420			Leu		425					430		
		435				Arg	440					445			
	450					Leu 455					460				
465		-			470	Lys				475				_	480
			_	485		Gly		_	490	_	_			495	
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PCT/US00/32919 WO 01/40474 164

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Val Val Ser Tyr Gly His Ile Lys His Asp Thr Thr Thr Leu Tyr Pro
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Gln Lys Gln Phe Thr Glu Ile Asp Tyr Asp Pro Arg His Phe Asp Asp
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Cys Ala Tyr Arg Asn Leu Ser Leu Pro Val Gly Cys Ala Val Glu Gly
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                                                                     420
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600

660

720

780

840

900

960

1020

1080

1140

1200

1260

1320

1380

1440

1464

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Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala
                            40
Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
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Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
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Arg Pro Leu Met Lys Phe Met Ser Ala Thr Ala Val Phe Ala Ala Val
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600

660

720

780

840

900

960

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Ile Ala Gly Gln Ile Lys Leu Pro Thr Val His Ile Gly Pro Thr Ala
                            40
                                               45
Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val
                        55
Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr
                    70
                                        75
Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr
Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser
                                105
Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr
                            120
                                                125
Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp
                       135
                                           140
Arg Pro Leu Asp Thr Gln Val Ser Glu Ser Pro Glu Ser Thr Pro Ser
                   150
                                        155
Pro Asp Asp Val Leu Gly Lys Gly Gly Gly Ile Tyr Thr Glu Lys Ser
                                   170
               165
Leu Thr Ile Thr Gly Ile Thr Gly Thr Ile Asp Phe Val Ser Asn Ile
                                185
Ala Thr Asp Ser Gly Ala Gly Val Phe Thr Lys Glu Asn Leu Ser Cys
                            200
Thr Asn Thr Asn Ser Leu Gln Phe Leu Lys Asn Ser Ala Gly Gln His
                       215
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Gly Gly Gly Ala Tyr Val Thr Gln Thr Met Ser Val Thr Asn Thr Thr

235

230

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Ser Glu Ser Ile Thr Thr Pro Pro Leu Val Gly Glu Val Ile Phe Ser
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             245
Glu Asn Thr Ala Lys Gly His Gly Gly Gly Ile Cys Thr Asn Lys Leu
                                  270
                         265
         260
Ser Leu Ser Asn Leu Lys Thr Val Thr Leu Thr Lys Asn Ser Ala Lys
                              285
      275 280
Glu Ser Gly Gly Ala Ile Phe Thr Asp Leu Ala Ser Ile Pro Thr Thr
                          300
                 295
Asp Thr Pro Glu Ser Ser Thr Pro Ser Ser Ser Pro Ala Ser Thr
                      315
              310
Pro Glu Val Val Ala Ser Ala Lys Ile Asn Arg Phe Phe Ala Ser Thr
                    330
            325
Ala Glu Pro Ala Ala Pro Ser Leu Thr Glu Ala Glu Ser Asp Gln Thr
                        345
Asp Gln Thr Glu Thr Ser Asp Thr Asn Ser Asp Ile Asp Val Ser Ile
                      360
Glu Asn Ile Leu Asn Val Ala Ile Asn Gln Asn Thr Ser Ala Lys Lys
                   375
Gly Gly Ala Ile Tyr Gly Lys Lys Ala Lys Leu Ser Arg Ile Asn Asn
               390 395
Leu Glu Leu Ser Gly Asn Ser Ser Gln Asp Val Gly Gly Leu Cys
           405 410
Leu Thr Glu Ser Val Glu Phe Asp Ala Ile Gly Ser Leu Leu Ser His
        420 425
Tyr Asn Ser Ala Ala Lys Glu Gly Gly Val Ile His Ser Lys Thr Val
  435 440
                              445
Thr Leu Ser Asn Leu Lys Ser Thr Phe Thr Phe Ala Asp Asn Thr Val
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          455
Lys Ala Ile Val Glu Ser Thr Pro Glu Ala Pro Glu Glu Ile Pro Pro
                                475 480
       470
Val Glu Gly Glu Glu Ser Thr Ala Thr Glu Asn Pro Asn Ser Asn Thr
              485 490
Glu Gly Ser Ser Ala Asn Thr Asn Leu Glu Gly Ser Gln Gly Asp Thr
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          500
                          505
 Ala Asp Thr Gly Thr Gly Val Val Asn Asn Glu Ser Gln Asp Thr Ser
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 Asp Thr Gly Asn Ala Glu Ser Gly Glu Gln Leu Gln Asp Ser Thr Gln
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 Ser Asn Glu Glu Asn Thr Leu Pro Asn Ser Ser Ile Asp Gln Ser Asn
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60 55 Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr 70 75 Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr 90 85 Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser 105 100 Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr 125 120 115 Leu Ala Glu Gly Pro Pro Ala Glu Phe Cys Arg Tyr Pro Ser His Trp 140 130 135 Arg Pro Leu Asp Gln Ser Asn Glu Asn Thr Asp Glu Ser Ser Asp Ser 150 155 · 160 His Thr Glu Glu Ile Thr Asp Glu Ser Val Ser Ser Ser Lys Ser 165 170 175 Gly Ser Ser Thr Pro Gln Asp Gly Gly Ala Ala Ser Ser Gly Ala Pro 190 180 185 Ser Gly Asp Gln Ser Ile Ser Ala Asn Ala Cys Leu Ala Lys Ser Tyr 205 200 Ala Ala Ser Thr Asp Ser Ser Pro Val Ser Asn Ser Ser Gly Ser Asp 210 215 Val Thr Ala Ser Ser Asp Asn Pro Asp Ser Ser Ser Ser Gly Asp Ser 230 235 Ala Gly Asp Ser Glu Gly Pro Thr Glu Pro Glu Ala Gly Ser Thr Thr 250 245 Glu Thr Pro Thr Leu Ile Gly Gly Gly Ala Ile Tyr Gly Glu Thr Val 260 265 Lys Ile Glu Asn Phe Ser Gly Gln Gly Ile Phe Ser Gly Asn Lys Ala 275 280 285 Ile Asp Asn Thr Thr Glu Gly Ser Ser Ser Lys Ser Asn Val Leu Gly 295 Gly Ala Val Tyr Ala Lys Thr Leu Phe Asn Leu Asp Ser Gly Ser Ser 315 310 Arg Arg Thr Val Thr Phe Ser Gly Asn Thr Val Ser Ser Gln Ser Thr 330 325 Thr Gly Gln Val Ala Gly Gly Ala Ile Tyr Ser Pro Thr Val Thr Ile 345 Ala Thr Pro Val Val Phe Ser Lys Asn Ser Ala Thr Asn Asn Ala Asn 360 Asn Ala Thr Asp Thr Gln Arg Lys Asp Thr Phe Gly Gly Ala Ile Gly 380 375 Ala Thr Ser Ala Val Ser Leu Ser Gly Gly Ala His Phe Leu Glu Asn 395 385 390 Val Ala Asp Leu Gly Ser Ala Ile Gly Leu Val Pro Asp Thr Gln Asn 410 405 Thr Glu Thr Val Lys Leu Glu Ser Gly Ser Tyr Tyr Phe Glu Lys Asn 425 420 Lys Ala Leu Lys Arg Ala Thr Ile Tyr Ala Pro Val Val Ser Ile Lys 445 440 Ala Tyr Thr Ala Thr Phe Asn Gln Asn Arg Ser Leu Glu Glu Gly Ser 435 460 455 Ala Ile Tyr Phe Thr Lys Glu Ala Ser Ile Glu Ser Leu Gly Ser Val 475 470 Leu Phe Thr Gly Asn Leu Val Thr Pro Thr Leu Ser Thr Thr Thr Glu 490 495 485 Gly Thr Pro Ala Thr Thr Ser Gly Asp Val Thr Lys Tyr Gly Ala Ala 505 Ile Phe Gly Gln Ile Ala Ser Ser Asn Gly Ser Gln Thr Asp Asn Leu

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Glu 545	Tyr	Arg	Pro	Thr	Ser 550	Ser	Asp	Thr	Gly	Thr 555	Ser	Thr	Phe	Cys	Ser 560
Ile	Ala	Gly	Asp	Val 565	Lys	Leu	Thr	Met	Gln 570	Ala	Ala	Lys	Gly	Lys 575	Thr
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Gln	Ala	Thr 595	Ala	Tyr	Asp	Thr	Leu 600	Asp	Ile	Asn	Lys	Ser 605	Glu	Asp	Ser
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Leu 625	His	Glu	Asn	Lys	Ser 630	Tyr	Ile	Pro	Gln	Asn 635	Val	Val	Leu	His	Ser 640
Gly	Ser	Leu	Val	Leu 645	Lys	Pro	Asn	Thr	Glu 650	Leu	His	Val	Ile	Ser 655	Phe
Glu	Gln	Lys	Glu 660	Gly	Ser	Ser	Leu	Val 665	Met	Thr	Pro	Gly	Ser 670	Val	Leu
Ser	Asn	Gln 675	Thr	Val	Ala	Asp	Gly 680	Ala	Leu	Val					

